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PB-271 659

Allergic Response to Platinum and Palladium Complexes Determination of No-Effect Level

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Prepared for

Health Effects Research Lab, Research Triangle Park, N C

Jul 77

PB 271 659

EPA-600/1-77-030
July 1977

Environmental Health Effects Research Series

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EPA-600/1-77-019
July 1977

ALLERGIC RESPONSE TO PLATINUM AND PALLADIUM COMPLEXES

DETERMINATION OF NO-EFFECT LEVEL

by

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The relationship of the following report to the overall objectives of the Health Effects Research Laboratory is based on the health risks associated with the potential release of noble metal attrition products into the atmosphere from the use and operation of catalytic converters. Principle components among these suspected attrition products include platinum and palladium compounds. Hypersensitivity has been a recognized occupational hygiene problem among workers in platinum refineries as well as a concern with the possible therapeutic use of organoplatinum compounds as anticancer agents.

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ABSTRACT

Section A

Rabbits, guinea pigs and mice were subcutaneously injected with $PtSO_4$ (with and without NH_4Cl) and $PdSO_4$ (with an without NH_4Cl) in an attempt to sensitise the animals to platinum or palladium. No allergic induction was found. Levels of platinum or palladium in the serum, urine and spleens of animals were monitored by AAS (atomic absorption spectrophotometer). Significant levels were found primarily in the urine of guinea pigs.

Section B

No allergic induction to platinum or palladium was found in rabbits, guinea pigs or mice when these animals were injected intravenously with platinum or palladium. Dermal contact with platinum was also tested on rabbits and guinea pigs but failed to induce an allergic state.

Platinum and palladium levels in the sera, urine and spleens of the animals, as monitored by AAS, were not significant.

Section C

Rabbits, guinea pigs and mice were intravenously or subcutaneously injected with a platinum-egg albumin complex or a palladium-egg albumin complex. Skin tests or footpad tests were performed 10-14 days after the last intravenous or subcutaneous injection. Only guinea pigs injected subcutaneously with palladium-egg albumin complex developed a hypersensitivity. This hypersensitivity to palladium was of the delayed type and was passively transferred to normal recipient guinea pigs via spleen cells from sensitive donors.

Section D

A delayed type allergy was induced in guinea pigs subcutaneously injected with palladium complexed to egg albumin. A minimum palladium concentration of 1.7 mg/ml is needed for induction. When guinea pig albumin is complexed to palladium a higher (5.0 mg/ml) palladium concentration is necessary.

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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1 REPORT NO. EPA-600/1-77-039	3 RECIPIENT'S ACCESSION NO.
4 TITLE AND SUBTITLE: Allergic Response to Platinum and Palladium Complexes - Determination of No-Effect Level	5 REPORT DATE: July 1977
	6 PERFORMING ORGANIZATION CODE
7 AUTHOR(S): James Taubler	8 PERFORMING ORGANIZATION REPORT NO.
9 PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Biology, St. Vincent College, Latrobe, Pennsylvania 15650	10 PROGRAM ELEMENT NO. 1AA601
	11 CONTRACT/GRANT NO. Grant No. R803036
12 SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711 RTP/NC	13 TYPE OF REPORT AND PERIOD COVERED
	14 SPONSORING AGENCY CODE EPA 600/11
15 SUPPLEMENTARY NOTES	

16 ABSTRACT

Section A - Rabbits, guinea pigs and mice were subcutaneously injected with $PtSO_4$ (with and without NH_4Cl) and $PdSO_4$ (with and without NH_4Cl) in an attempt to sensitize the animals to platinum or palladium. No allergic induction was found. Levels of platinum or palladium in the serum, urine and spleens of animals were monitored by AAS (Atomic absorption spectrophotometer). Significant levels were found primarily in the urine of guinea pigs.

Section B - No allergic induction to platinum or palladium was found in rabbits, guinea pigs or mice when these animals were injected intravenously with platinum or palladium. Dermal contact with platinum was also tested on rabbits and guinea pigs but failed to induce an allergic state. Platinum and palladium levels in the sera, urine and spleens of the animals, as monitored by AAS, were not significant.

Section C - Rabbits, guinea pigs and mice were intravenously or subcutaneously injected with a platinum-egg albumin complex or a palladium-egg albumin complex. Skin tests or footpad tests were performed 10-14 days after the last intravenous or subcutaneous injection. Only guinea pigs injected subcutaneously with palladium-egg albumin complex developed a hypersensitivity of the delayed type.

Section D - A delayed type allergy was induced in guinea pigs subcutaneously injected with palladium complexed to egg albumin.

17 KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c COSATI Field/Group
platinum allergic diseases palladium		06 T

18 DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19 SECURITY CLASS (This Report) UNCLASSIFIED	21 NO. OF PAGES 80
	20 SECURITY CLASS (This page) UNCLASSIFIED	22 PRICE NTIS - A01

EPA Form 2220-1 (9-73)

INTRODUCTION

The immunological phenomena of man and many other lower animals that have evolved during thousands of centuries can be arbitrarily categorized in two groups, i.e., those that are protective and those that are harmful. At man's present level of knowledge the protective immunological phenomena far outnumber the harmful, however, this may be due to the subtle obscure nature of the immunological phenomena involved in producing harmful effects. Hypersensitivity or allergy is the term used to denote an immunological reaction that is harmful to the host within which it occurs. Hypersensitivity can be classified into two types, immediate and delayed. The essential difference between the two is the type of immune response, i.e., the immediate is mediated thru a humoral (antibody) immune response, the delayed thru a cellular immune response. Delayed hypersensitivity or delayed allergy also requires a longer time to develop ranging from 6 - 24 hours after challenge whereas the immediate allergy is usually manifested within the first hour.

Immediate Allergy:

Immediate allergy or hypersensitivity involves the sensitization of the host by an allergen (antigen). The allergens of hay fever can be obtained from a wide variety of material such as pollen, grasses (hay, ragweed, golden rod, lawn grasses, etc.) and animal dander. The antigen involved in the production of hives can be obtained from almost any food but in particular foods such as fish, milk, chocolate and fruit. Asthma and anaphylaxis are also examples of immediate allergy; anaphylaxis, being the most severe of all allergic reactions, often can be fatal.

Allergens that by themselves can induce the allergic state are usually complex macromolecules. However, low molecular weight substances such as penicillin, certain chemical compounds and indeed even elements can complex with large molecular weight host proteins and induce the hypersensitive state. Platinum is an example of an element that can induce immediate type allergies. Little is known about platinum allergies but from the few reports concerning platinum allergies(1 - 6), it appears that platinum can induce an asthma and a dermatological(rash) type reaction. It should be added that simple chemicals are usually themselves not allergens but act as haptenes. A hapten can combine with large molecules (usually proteins) and induce allergies specific for the hapten.

Delayed allergy:

Delayed hypersensitivity or delayed allergy is also known as infection allergy since for many years it could only be demonstrated subsequent to infection (i.e., certain viral diseases and intracellular bacterial diseases such as tuberculosis and brucellosis).

Immune stimulation by antigens (usually virons, bacterial cells or chemicals) is required to induce delayed allergy. Upon reexposure to the antigen the sensitized lymphoid cells are injured. The mechanism of injury is ill defined; nevertheless, delayed allergy can be demonstrated by means of various in vivo or in vitro tests. Smallpox vaccination, the various tuberculin tests and brucellin tests are all manifestations of delayed allergy. Additionally poison ivy rash and the dermatological reactions to various chemicals, some of which are carcinogens are all forms of allergy.

Whether simple elements such as platinum or palladium can induce delayed allergy is unknown. However, simple haptenes such as penicillin can induce delayed allergy thus, delayed allergy to metals such as platinum and palladium may indeed be probable. Apparently a prime criterion for antigenicity of a small molecular weight substance (i.e., mercury or platinum) is the ability of the metal to form complexes with skin or serum protein, most likely by reacting with free amino or sulphydryl groups on the protein to form stable complexes.

Immediate and delayed allergy, which include numerous autoimmune diseases are but two manifestation of the immunological capabilities of man spanning such diverse phenomena as general immunity, tumor immunity and transplantation immunity. Basic research on the possible allergic reactions of metals such as platinum and palladium will not only provide environmental benefits but also aid in understanding the complex nature of man's immunological capacity.

CONCLUSIONS

Section A

Rabbits, guinea pigs and mice which were subcutaneously injected three times a week for 4 weeks with various concentrations of platinum and palladium salts showed:

1) no induction of an allergic state to platinum or palladium
2) significant palladium levels in the urine of rabbits and guinea pigs
3) significant platinum levels in the splenic tissue of rabbits and guinea pigs.

Section B

Rabbits, guinea pigs and mice which were intravenously injected three times a week for 4 weeks with various concentrations of platinum and palladium salts showed:

1) no induction of an allergic state to platinum or palladium
2) no significant platinum or palladium levels in the urine, serum or splenic tissue.

Also, rabbits and guinea pigs which were exposed to platinum paste (dermal contact with platinum paste) for 5 weeks showed:

1) no allergic induction
2) no significant levels of platinum in the urine, serum or splenic tissue.

Section C

Rabbits, guinea pigs and mice which were intravenously injected three times a week for 3 weeks with various concentrations of palladium - egg albumin complex showed:

1) no allergic induction
2) no significant palladium levels in the serum
3) significant palladium levels in the urine of guinea pigs
4) no significant palladium levels in the splenic tissues.

Guinea pigs and mice which were subcutaneously injected three times a week for 4 weeks with various concentrations of a palladium egg albumin complex showed:

1) a delayed allergy to palladium in guinea pigs
2) transfer of the palladium delayed allergy via spleen cells
3) no significant palladium levels in serum of guinea pigs
4) significant palladium levels in the urine of guinea pigs
5) no induced allergy in mice.

Rabbits and guinea pigs which were subcutaneously injected three times a week for 4 weeks with various concentrations of platinum-egg albumin complex showed:

1) no induced allergy
2) significant platinum levels in the serum of rabbits
3) significant platinum levels in both the urine and serum of guinea pigs.

Section D

Guinea pigs which were subcutaneously injected three times a week for 4 weeks with various concentrations of $PtCl_4$ or Pt^{+4}-albumin (guinea pig or egg) complex showed no allergic induction.

Guinea pigs which were subcutaneously injected three times a week for 4 weeks with various concentrations of $PdCl_4$ complexed to guinea pig or egg albumin showed:

1) induction of a delayed allergy
2) that 3.7 mg/ml of palladium complexed to egg albumin is the minimum concentration of palladium necessary for allergic induction
3) that higher palladium levels when complexed to guinea pig albumin will induce a delayed allergy
4) no immediate type allergy.

Although platinum allergies of the immediate type have been reported (1-8), no such allergic induction (immediate or delayed) has been demonstrated under the experimental conditions of this report. However, palladium when complexed to guinea pig or egg albumin will induce a delayed type allergy. As of this time, no report of palladium allergy is known except that demonstrated in this report.

SECTION A

SUBCUTANEOUS INJECTIONS

The following is a series of experiments (preliminary) to determine dose, the route of exposure and animal species needed to develop a test system for the allergic induction of platinum and palladium.

MATERIALS AND METHODS (GENERAL)

1) Animals
   a) Rabbits - all experimental rabbits used were 5 lb. albino females.
   b) Guinea pigs - all experimental guinea pigs used were 300 - 350 gram albino females.
   c) Mice - all experimental mice used were 20 - 30 gram white Swiss females.

2) Determination of platinum and palladium concentration
   a) Standardization of AAS (atomic absorption spectrophotometer) for platinum or palladium in sera
   A known serum sample containing 1.0 ml serum (to avoid matrix interference), 2.0 ml $LaCl_3$ and 7.0 ml platinum or palladium solution (various amounts) for a total of 10.0 ml was assayed against a serum blank containing 1.0 ml serum, 2.0 ml $LaCl_3$ and 7.0 ml H_2O to avoid serum adsorption. Solutions containing 1 ppm or more recovery were 99.7% for platinum and 96% for palladium, based upon 140 determinations.

   b) Standardization of AAS for platinum or palladium in urine
   A known urine sample containing 1.0 ml urine 2.0 ml $LaCl_3$ and 7.0 ml of platinum or palladium (various amounts) for a 10.0 ml total was assayed against a urine blank containing 1.0 ml urine, 2.0 ml $LaCl_3$ and 7.0 ml H_2O. In solutions containing 1 ppm or more, recovery was 90% for platinum and 95% for palladium based upon 140 determinations.

c) Determination of palladium and platinum in urine and serum
Serum and urine samples were assayed by AAS against known serum and urine samples.

d) Determination of palladium and platinum in spleen
Spleens were removed and weighed and cell extracts were assayed by AAS for platinum or palladium concentrations. The concentrations were recorded as mg metal/spleen mass.

Determination of allergic induction

a) Skin test - guinea pigs and rabbits
The flanks of the animals were shaved and the hair removed by a depilatory agent two days prior to the skin testing.
All skin tests were carried out 10 - 14 days after the last exposure to platinum or palladium. 0.1 ml of the platinum or palladium solution was injected intradermally. Test sites were examined for erythema and/or induration 1, 1, 2, 9, 18, 24 and 48 hours later.

b) Passive transfer
A spleen cell suspension in Hank's solution was used for transfer. A minimum of 5×10^8 cells were used for transfer. Cell counts were made with a hemocytometer. Twenty-four hours after transfer the recipient animals were skin tested.

c) Footpad test in mice
Mice were footpad tested with 0.1 ml of the various concentrations of $PtSO_4$ or $PdSO_4$. Saline was injected in the opposite foot as a control. Caliper reading was used to determine if a positive footpad test did occur. The net swelling in the test foot at 24 hr (difference in measurement between 24 hr reading and pre-injection reading) is compared to the net swelling in the control foot at 24 hr (difference in measurement between 24 hr reading and pre-injection reading). This net swelling is due to the metal ($PtSO_4$ or $PdSO_4$). Normal mice were footpad tested using the same procedure.

EXPERIMENTS AND RESULTS

Experiment I

Fifteen rabbits and 15 guinea pigs were separated into 3 groups (5 animals/group) and subcutaneously injected with 1.0 ml of $PdSO_4$ in 0.3, 0.1, 0.05 mg/ml concentrations three times weekly for four weeks. The palladium solution also contained 0.2, 0.07 and 0.01 g/ml of NH_4Cl (the result of attempts to increase the solubility of $PdSO_4$).

Results

1) Skin Test

In addition to the 3 concentrations of $PdSO_4$, all surviving animals were injected intradermally with 0.1 ml of 0.21 g/ml of NH_4Cl as a control. All sites showed a 1 to 1.5 cm diameter swelling at 30 min and 1 hr. The swelling was gone at 2 hr and a necrotic area with erythema occurred at all sites at 24 hr.

2) Palladium levels in sera and urine of guinea pigs and rabbits

Tables 1 and 2 list palladium levels in urine and serum of individual guinea pigs injected subcutaneously with various concentrations of $PdSO_4$. Tables 3 and 4 list the palladium levels in the urine and serum of individual rabbits injected subcutaneously with various concentrations of $PdSO_4$.

3) Palladium levels in spleens

No palladium was detected in the spleens of guinea pigs or rabbits.

Discussion of Results

1) Guinea pigs

From Table 1 it can be seen that guinea pigs injected subcutaneously with 1.0 ml of a 0.3 mg/ml $PdSO_4$ solution showed significant levels of palladium in the urine at 4 days. The palladium levels after this are of limited value since they are only from one animal. In pigs receiving 0.1 mg/ml dose of $PdSO_4$ the palladium concentration in the urine steadily rose to a high point at 18-25 days after which all of the animals died. Pigs receiving the lowest dose of $PdSO_4$ (0.05 mg/ml) showed a decline in palladium in the urine after a higher 4 day level. Then a second increase occurred at 25 days.

All the animals (except one) died from the injections, which was unexpected. This unexpected result deserves the following comments. There was marked and extensive necrosis at the injection site (hip) and after 4-6 days paralysis of the hind limb was observed. Death occurred within 9 days with the highest dose. The highest dose of $PdSO_4$ was 3 mg/ml which also contained 0.21 g/ml of NH_4Cl. (The NH_4Cl was there because of attempts to increase the solubility of $PdSO_4$.) This high salt (NH_4Cl) concentration most likely would cause osmotic effects (cell damage and permeability changes) which could damage or cause malfunction of the sciatic nerve.

Similar conditions were observed in pigs injected with 0.1 mg/ml $PdSO_4$ in 0.07 g/ml NH_4Cl and 0.05 mg/ml $PdSO_4$ in 0.035 g/ml NH_4Cl, although paralysis and death were delayed. Was death due to the $PdSO_4$ or the NH_4Cl or both? To obtain some insight, five pigs were injected with 1.0 ml of a 0.2 g/ml NH_4Cl solution. The results were strikingly similar to those observed in pigs receiving both NH_4Cl and $PdSO_4$. Paralysis and death occurred within 5 days. Thus it would appear that the high salt concentration is toxic enough to cause death in pigs. It should be noted that the larger (by weight) the pig the longer it survived; thus body weight - dose relationship appears to be involved. This is supported by the fact that no rabbits (much higher weight - dose relationship) died due to the injection. However, necrosis at the injection site was observed.

With extensive tissue damage (evident by large necrotic area) it would seem reasonable that the palladium would enter the blood stream and be filtered by the kidney which would explain the palladium levels in the urine. However, from Table 2 it is obvious that no significant level of palladium in the serum occurred. How palladium levels occur in the urine and not in the blood is unresolved at present.

3) Rabbits
Examination of Table 3 shows that the highest palladium level in the urine was found in rabbits injected with the high dose (0.3 mg/ml). The lower doses, 0.1 and 0.05 mg/ml, showed lower palladium urine levels. In all animals, however, the palladium levels increased up to the 18th day of the experiment. Table 4 demonstrates no significant palladium serum levels throughout the experiment.

Although there was necrosis at the injection site (hip) it was less extensive than that observed in the pigs. Also, no rabbits died from the injection; the rabbit deaths that did occur were the result of blood sampling (cardiac puncture).

3) Skin Tests
All tests were negative.

4) Palladium levels in splenic tissue
No palladium levels were found.

EXPERIMENT II
Fifteen rabbits, guinea pigs and mice were separated into three groups (5 animals/group) and subcutaneously injected with 1.0 ml of $PdSO_4$ in the following concentrations: 0.35, 0.10 and 0.05 mg/ml three times weekly for four weeks.

Results

1) Skin test
No edema or erythema occurred.

2) Footpad test in mice
All were negative.

3) Platinum levels in urine and sera of guinea pigs and rabbits
Tables 4 and 5 list platinum levels in the urine and serum of individual guinea pigs injected subcutaneously with various concentrations of $PtSO_4$.
Tables 7 and 8 list platinum levels in the urine and serum of individual rabbits injected subcutaneously with various concentrations of $PtSO_4$.

4) Platinum concentration in splenic tissues
Table 9 shows the average of $PtSO_4$ concentration in splenic tissue of guinea pigs, rabbits and mice subcutaneously injected with various $PtSO_4$ concentrations.

Discussion of Results

1) $PtSO_4$ in urine of guinea pigs and rabbits
Significant levels of $PtSO_4$ were found in the urine of guinea pigs at only one point; i.e., in guinea pigs injected with the highest concentration of $PtSO_4$ (0.35 mg/ml), the level of $PtSO_4$ in the urine at 21 days reached 0.002 mg/ml. This is quite low, but significant. No significant levels of $PtSO_4$ were found in the urine of rabbits.

2) $PtSO_4$ in serum of guinea pigs and rabbits
No significant levels of $PtSO_4$ were found in the serum of guinea pigs or rabbits.

3) Skin Test
A small area of necrosis occurred at the skin test site with the highest concentration of $PtSO_4$ (0.1 ml of a 0.35 mg/ml conc). This proved to be a toxic reaction and not an allergic one, since normal animals also show a similar response. Apparently a 0.1 ml of a 0.35 mg/ml $PtSO_4$ solution injected intradermally is toxic to the dermis.

4) Uptake of $PtSO_4$ by the spleen in guinea pigs, rabbits and mice
Table 9 lists the amounts of $PtSO_4$ concentrated in the spleens of the 3 species of animals. The amounts are comparable since the $PtSO_4$ concentrations are given in mg/gm spleen weight. In animals injected with the high conc (0.35 mg/ml) of $PtSO_4$, rabbit spleens picked up approximately 0.016 mg/gm, guinea pig spleens 0.050 mg/gm and mouse spleens 0.141 mg/gm. This suggests roughly a 3-fold increase from rabbits to guinea pigs to mice; i.e., rabbit spleens pick up X, guinea pigs 3X and mice 10X. The large $PtSO_4$ conc in mouse spleens as compared to the rabbit spleens is most likely due to the relative size of the spleens of each species, the rabbit spleen being approximately 6-7 times as large (by weight) as that of the mouse. The guinea pig spleen is approximately midway (regarding weight) between that of the rabbit and the mouse. The significance of $PtSO_4$ concentration in the spleens of various species is questionable at this point, but may be critical in the over-all immunology and physiology of a species if a certain concentration would be shown to have toxic effects.

5) Mouse footpad tests
Test animals (animals injected with $PtSO_4$) and normal animals gave similar footpad test reactions.

CONCLUSIONS

1) Generally no significant levels of $PtSO_4$ were found in the urine of guinea pigs and rabbits.

2) No significant levels of $PtSO_4$ were found in the serum of guinea pigs and rabbits.

3) No immediate or delayed type allergy was induced in guinea pigs, rabbits or mice.

4) All skin tests in guinea pigs and rabbits and all footpad tests in mice were negative.

5) Considerable levels of $PtSO_4$ were found in the spleens of the three species. The largest amounts of $PtSO_4$ were found in the mouse spleens, the least in the rabbit spleens.

COMPARISON OF EXPERIMENTS I AND II

Urine levels of palladium and platinum in rabbits and guinea pigs

1) Injected with 0.30 mg/ml $PdSO_4$ and 0.35 mg/ml $PtSO_4$

Rabbits - Significant levels of palladium were found in the urine of rabbits while no significant levels of platinum were found.

Maximum levels of palladium occurred at 18 days and reached 0.0047 mg/ml, while the maximum level of platinum occurred at 7 days being 0.0012 mg/ml, a reading of doubtful significance.

Guinea pigs - Significant levels of palladium were found in the urine of guinea pigs, maximum levels occurring at 4 days and reading 0.021 mg/ml. A low but significant level of platinum occurred at 21 days, reading only 0.002 mg/ml.

2) Injected with 0.1 mg/ml $PdSO_4$ and 0.1 mg/ml $PtSO_4$

Rabbits - Significant levels of palladium were found in the urine of rabbits while no significant level of platinum was found. Maximum levels of palladium occurred at 4 days (0.0015 mg/ml).

Guinea pigs - Significant levels of palladium were found throughout the experimental period, reaching a maximum level of 0.0117 mg/ml at 25 days. No significant levels of platinum were found in the urine of guinea pigs.

3) Injected with 0.05 mg/ml $PdSO_4$ and 0.05 mg/ml $PtSO_4$

Rabbits - Significant levels of palladium were found in the urine. Maximum level was 0.0015 mg/ml at 4 days. No significant levels of platinum were found in the urine of rabbits.

Guinea pigs - Significant levels of palladium were found throughout the experimental period, reaching a maximum level of 0.0084 mg/ml at 28 days. No significant levels of platinum were found.

4) Spleen levels of palladium and platinum in rabbits and guinea pigs

No significant levels of palladium were found. High levels of platinum were found, which suggests that platinum has a much greater affinity for splenic tissue than palladium.

Table 10 lists maximum levels of palladium and platinum in the urine of guinea pigs and rabbits.

The following statements regarding palladium and platinum levels in the urine of rabbits and guinea pigs can be made.

1) Much more palladium enters the urine of rabbits and guinea pigs than platinum.

2) Much more palladium enters the urine of guinea pigs than rabbits.

3) The levels of platinum are below significance for both rabbits and guinea pigs.

EXPERIMENT III

The purpose of this experiment is to determine the effect of NH_4Cl on platinum levels in urine, sera and spleens. Nine rabbits and guinea pigs were separated into 3 groups (3 animals/group) and subcutaneously injected with 1.0 ml of 0.35 mg $PtSO_4$/ml and 0.2 g NH_4Cl/ml, 0.1 mg $PtSO_4$/ml and 0.07 g NH_4/ml, and 0.05 mg $PtSO_4$/ml and 0.03 g NH_4Cl/ml three times weekly for four weeks.

Results

1) Platinum levels in urine and sera of guinea pigs and rabbits
Tables 11 and 12 list the platinum levels in serum and urine of individual rabbits injected subcutaneously with various concentrations of $PtSO_4$ and NH_4Cl.

Tables 13 and 14 list the platinum levels in serum and urine of individual guinea pigs injected subcutaneously with various concentrations of $PtSO_4$ and NH_4Cl.

2) Platinum concentration in splenic tissues
No platinum was detected in the spleens of the guinea pigs and rabbits.

EXPERIMENT IV

Fifteen rabbits and guinea pigs were separated into three groups (5 animals/group) and subcutaneously injected with 1.0 ml of 0.1 mg, 0.1 mg and 0.05 mg/ml $PdSO_4$ three times weekly for four weeks.

Results

Tables 15, 16, 17 and 18 show no significant levels of palladium in the urine or sera of guinea pigs or rabbits.

All skin tests were negative.

Discussion

Comparison of the subcutaneously injected $PdSO_4$ and $PtSO_4$ (with and without NH_4Cl) experiments

Tables 19 and 20 give the comparative palladium and platinum levels in the sera and urine of rabbits and guinea pigs. The following statements can be made regarding palladium and platinum in urine and sera of rabbits and guinea pigs.

1) Sera

Rabbits: Minimal significant levels of $PtSO_4$ occurred only when NH_4Cl was injected with the $PtSO_4$. No significant levels of $PdSO_4$ were detected.

Guinea pigs: No significant levels of $PtSO_4$ or $PdSO_4$ were found. All pigs died, however, with NH_4Cl experiment.

2) Urine

Rabbits: Significant levels of palladium occurred in urine and higher levels when NH_4Cl is used. No significant levels of platinum were found.

Guinea pigs: Significant levels of palladium occurred in urine, higher when NH_4Cl is used. No significant levels of platinum were found.

Note: It does appear that NH_4Cl caused higher levels of palladium to be found in the urine.

3) Spleen

Significant levels of platinum were found in the spleens of rabbits and guinea pigs only when $PtSO_4$ was injected alone. In experiments where $PtSO_4$ plus NH_4Cl were injected no significant platinum levels could be found. Thus it appears that platinum can be taken up in the spleens of rabbits and guinea pigs and NH_4Cl can inhibit this uptake. Palladium was never found in the spleens of rabbits or guinea pigs.

SECTION B

INTRAVENOUS AND DERMAL CONTACT

The following experiments (preliminary) were used to determine if the intravenous or dermal contact with platinum or palladium would induce an allergic response.

MATERIALS AND METHODS

See Section A.

EXPERIMENTS AND RESULTS

EXPERIMENT V

Fifteen rabbits were separated into three groups (5 animals/group) and injected intravenously (marginal ear vein) three times a week for three weeks with 1.0 ml of a 0.35 mg, 0.10 mg and 0.05 mg/ml $PtSO_4$ solution.

Forty-five mice were separated into three groups (15 mice/group) and injected intravenously (tail vein) three times a week for three weeks with 0.25 ml of a 0.35 mg, 0.10 mg and 0.05 mg/ml $PtSO_4$.

Rabbit skin testing was done 14 days after the last intravenous injection by injecting 0.1 ml of the 3 $PtSO_4$ concentrations intradermally. Mice were footpad tested 14 days after the last intravenous injection by injecting 0.05 ml of 3 $PtSO_4$ concentrations into the hind footpads.

Results

All rabbit skin tests and mouse footpad tests were negative. Tables 21 and 22 list the $PtSO_4$ levels in the urine and serum of individual rabbits injected intravenously with various concentrations of $PtSO_4$. No significant levels were found. Note: Mouse serum and urine were not used since sufficient quantities cannot be obtained.

Conclusions

1) No allergic induction in rabbits or mice injected intravenously with $PdSO_4$.
2) No significant levels of $PdSO_4$ in the urine or sera of rabbits. (Mouse serum and urine were not used since sufficient quantities cannot be obtained.

EXPERIMENT VI

Fifteen rabbits (5 rabbits/group), 15 guinea pigs (5 guinea pigs/group) and 45 mice (15 mice/group) were separated into 3 groups and injected intravenously. Rabbits and guinea pigs received 1.0 ml of 0.35 mg, 0.10 mg and 0.05 mg/ml $PdSO_4$ three times a week for 3 weeks. Mice received 0.25 ml instead of 1.0 ml. The intravenous injections in rabbits were via the marginal ear vein, in guinea pigs the dorsal saphenous vein and in mice the ventral tail vein were injected.

Skin and footpad tests were done 12 days after the last intravenous injection.

Results

1) All skin and footpad tests were negative. Note: In the case of the guinea pig, the dorsal saphenous vein collapses and an extreme hematoma occurs, making subsequent intravenous injection impossible. The hematoma most likely is due to the toxic nature of $PdSO_4$. (This toxic effect is also seen in skin test sites where necrosis of the dermis is seen.) Thus guinea pigs received only 5 of the scheduled 9 injections. Nevertheless, they were skin tested along with the rabbits and all sites were negative.
2) There were no detectable palladium levels in the animal spleens.
3) There were no detectable levels of $PdSO_4$ in the urine or sera of rabbits or guinea pigs.

Conclusions

1) There was no induction of allergy in rabbits, guinea pigs or mice.
2) No $PdSO_4$ was found in urine, sera, spleen or kidney in any of the three species.
3) The results of this experiment are similar to those of the $PdSO_4$ (i.v.) experiment, i.e., no metal was found in any of the tissues or urine examined.
4) The guinea pig does not appear to be a good experimental animal for intravenous injections of platinum or palladium since these metals are toxic, causing necrosis in the leg area (injection site).

EXPERIMENT VII
Platinum Paste Experiment

Fourteen rabbits and 15 guinea pigs were exposed to platinum (7 rabbits exposed to 0.25 gm/application and 7 exposed to 0.1 gm/application; 8 guinea pigs exposed to 0.25 gm/application and 7 exposed to 0.1 gm/application) via a paste (H_2O and $PtSO_4$). The paste was applied to the flank of each animal and held in position by gauze and tape. (Prior to the paste application the hair of each animal was removed so that the bare skin was in direct contact with the metal.) Fresh applications were made each week for 5 weeks. Fourteen days after the last application of platinum paste the animals were skin tested with various concentrations of $PtSO_4$.

Results

1) Skin test - All surviving* animals were skin tested 14 days after last application of platinum paste.

   Skin test procedure: 0.1 ml of a 0.35 mg/ml, 0.1 mg/ml or 0.05 mg/ml $PtSO_4$ solution was injected intradermally. As a control 0.1 ml of a saline solution was intradermally injected. Sites were examined 30 min, 1 and 2 hrs, and 1 and 2 days later. All guinea pigs gave a negative skin test. Six rabbits (1 exposed to 0.25 gm/application and 4 exposed to 0.1 gm/application) produced a questionable skin reaction upon skin testing. A description of the skin test reactions in these rabbits follows: No reaction at 30 min or 1 hr. At 24 hrs some swelling with mild erythema. Less swelling at 48 hrs with faint erythema. At 3-4 days necrosis at the platinum site.

   Normal rabbits produce the same reactions, suggesting that the reaction was not allergically mediated, but due to toxic effects of platinum. Nevertheless 2 rabbits (those giving the best reaction on skin testing) were skin tested again with a 1/50 dilution of the 0.05 mg/ml $PtSO_4$ solution. The rationale for injecting the 1/50 dilution of the 0.05 mg/ml $PtSO_4$ was to determine whether the reaction was due to toxicity or allergy. Again a small area of necrosis occurred at the test site in these and normal rabbits. Thus it was concluded that the skin test results were negative. Additionally, serum and spleen cells from these 2 rabbits were used in an attempted passive transfer for immediate and delayed allergy. The results for both were negative.

   *Deaths in animals before sacrificing were due to bleeding and not due to exposure to platinum paste (cardiac puncture).

2) After skin testing all animals were sacrificed (except those used in attempted passive transfer) and the $PtSO_4$ levels in the splenic tissue assessed. No significant levels of $PtSO_4$ in the spleens of guinea pigs or rabbits exposed to platinum paste were found.

3) Concentration of $PtSO_4$ in urine and serum of rabbits and guinea pigs exposed to the platinum paste - Samples were taken once a week for 5 weeks and no significant levels of $PtSO_4$ were found. (Tables 23 - 26)

Conclusions

1) No induction of immediate or delayed allergy by exposure of guinea pigs or rabbits to platinum paste was observed.
2) No significant levels of $PtSO_4$ in urine or serum of guinea pigs or rabbits exposed to platinum paste were noted.
3) No significant levels of $PtSO_4$ were found in the spleens of rabbits or guinea pigs exposed to platinum paste.

SECTION C

METAL ALBUMIN COMPLEX

Since the subcutaneous, intravenous and dermal exposure routes to palladium and platinum appear not to induce an allergic state, an alternate approach was taken. Allergies to metals do exist and in many cases are induced by the metal complexing to skin or blood proteins. Based on this rationale, experiments were designed to determine the ability of a palladium or a platinum complex to induce the allergic state.

MATERIALS AND METHODS

The materials and methods are basically the same as in Sections A and B, with the following addition:

Procedure for complexing palladium to albumin

A stock solution of palladium-albumin complex is prepared in the following manner:

> One gram of Na_2PdCl_4 is dissolved in 5 ml phosphate buffer pH7.0 and allowed to set for 18 hours. This solution is autoclaved, and then 5 ml of a 1 g/50 ml solution of albumin is added (total amount of albumin added is 100 mg) and allowed to set overnight. The pH is adjusted to 7.0 with 2.0 M NaOH and the palladium content determined by AAS. This Pd-alb complex solution is centrifuged and the supernatant is checked for the presence of chlorine by adding $AgNO_3$ (0.1 g $AgNO_3$ and 5 ml H_2O). A white precipitate forms and the solution centrifuged. The supernatant is discarded and the precipitate washed with H_2O. H_2O and concentrated NH_4OH are then added to the precipitate which dissolves, indicating the presence of chloride. The presence of the protein was confirmed by the nitrous acid test.
>
> Working solutions of the palladium-albumin complex were prepared by dilutions of the stock solutions.

EXPERIMENT VIII

Palladium/Egg Albumin Complex (Intravenous Route)

Fifteen rabbits and 15 guinea pigs were separated into three groups (5 animals/group) and injected intravenously with 1.0 ml of 10 mg Pd and 1.5 mg egg albumin/ml, 1 mg Pd and 0.15 mg egg albumin/ml and 0.1 mg Pd and 0.05 mg egg albumin/ml three times per week for 3 weeks. Rabbits and guinea pigs were skin tested 10 days after the last intravenous injection by an intradermal

injection of 0.1 ml of the three palladium/albumin complexed solutions. (Note: after 4 intravenous injections to guinea pigs, it was necessary to change to the subcutaneous route due to the collapse of the saphenous vein. Thus guinea pigs had 4 intravenous and 5 subcutaneous injections. Forty-five mice were separated into 3 groups (15 mice/group) and injected intravenously with 0.25 ml of each of the 3 palladium/egg albumin complex solutions. Mice were footpad tested 10 days after the last intravenous injection.

Results

1) Palladium Urine Levels
Tables 27 and 28 list the urine levels of palladium in rabbits and guinea pigs. Significant levels were attained only in guinea pigs receiving 10 mg palladium. All serum readings were zero for both species. In rabbits all skin tests were negative.

2) Skin Test
Skin test measurements in guinea pigs are shown in Table 29. Positive skin reactions were found in guinea pigs receiving all 3 doses. However, passive transfer was not successful.

3) Footpad Test
Thirty of the 45 mice died before being footpad tested. The concentration of palladium used apparently is toxic. Breakdown of deaths is as follows:

13/15 died when injected with high concentration
(2.5 mg Pd and 0.4 mg albumin)

10/15 died when injected with middle concentration
(0.25 mg Pd and 0.04 mg albumin)

7/15 died when injected with low concentration
(0.08 mg Pd and 0.01 mg albumin)

The surviving mice (15) were footpad tested, but demonstrated no allergic induction.

4) Palladium Spleen Level
Palladium was not found in the spleens of any of the species.

CONCLUSIONS

1) There was no allergic induction in rabbits, guinea pigs or mice injected intravenously with various concentrations of palladium complexed to albumin three times per week for 3 weeks.

Note: The intravenous injections were administered to guinea pigs only 4 times (8 days), followed by subcutaneous injections. When these pigs were skin tested they had developed a delayed reaction (delayed hypersensitivity, Table 29). However, this "sensitivity" could not be transferred to normal recipients and therefore could not be considered a bona fide allergic

reaction. The pigs that experienced the apparent delayed reaction were injected subcutaneously for less than 2 weeks. Thus, they may not have had enough time to develop a full hypersensitivity. From the results of the second palladium-albumin complex experiment (2nd part of this report), this appears to be the explanation.

2) Palladium levels in urine and sera of animals

(a) Rabbits - no significant palladium levels in either sera or urine of rabbits injected intravenously with palladium-albumin complex

(b) Guinea pigs - no significant palladium levels in the sera of guinea pigs injected intravenously (3 times) and subcutaneously (5 times) with palladium-albumin complex

(c) Guinea pigs - significant palladium levels found in urine of pigs injected (3 times I.V. and 5 times s.c.) with 10 mg Pd-1.5 mg albumin complex

3) Palladium levels in spleen, kidney and liver of the three species

No significant palladium levels were found in these tissues when the3 species were injected intravenously with the palladium-albumin complex.

EXPERIMENT IX

Palladium/Egg Albumin Complex (Intravenously in Rabbits and Subcutaneously in Guinea Pigs)

This experiment was designed to recheck the negative results (no allergic induction) obtained when rabbits were intravenously injected with the palladium/egg albumin complex. Also, it was performed to check on the induction of an allergic state by egg albumin alone, using this immunizing schedule. Since questionable results were obtained in guinea pigs when part of the injection was subcutaneous, the subcutaneous route was chosen here for guinea pigs.

Procedure:

Guinea pigs were injected subcutaneously with 1.0 ml 3 times a week for 2 weeks with the following solutions:

2 rabbits received 10 mg Pd and 1.5 mg alb/ml
2 rabbits received 1 mg Pd and 0.15 mg alb/ml
1 rabbit received 0.1 mg Pd and 0.05 mg alb/ml
1 rabbit received 1.5 mg alb/ml
1 rabbit received 0.15 mg alb/ml

Guinea pigs were injected subcutaneously with 1.0 ml 3 times a week for 2 weeks with the following solutions:

2 guinea pigs received 10 mg Pd and 1.5 mg alb/ml
2 guinea pigs received 1 mg Pd and 0.15 mg alb/ml

3 guinea pigs received 0.3 mg Pd and 0.05 mg alb/ml
4 guinea pigs received 1.5 mg alb
3 guinea pigs received 0.15 mg alb

Mice were injected intravenously with 0.05 ml 3 times per week for 3 weeks with the following solutions:

9 mice received 1.5 mg alb
9 mice received 0.15 mg alb
9 mice received 10 mg Pd and 1.5 mg alb
9 mice received 1 mg Pd and 0.15 mg alb
9 mice received 0.3 mg Pd and 0.05 mg alb

Results

1) Allergic induction to palladium or albumin occurred.

2) There were no significant levels of palladium in sera or urine of rabbits. See Table 30.

3) No significant levels of palladium were found in the liver, kidney or spleens of rabbits.

4) Skin test results for individual guinea pigs are listed in Table 31 which shows a delayed hypersensitivity to the palladium-albumin complex developed in guinea pigs subcutaneously injected with the complex. Passive transfer was effected via spleen cells as seen from Table 32.

5) An immediate hypersensitivity to albumin developed in guinea pigs subcutaneously injected with albumin as seen from Table 31 (guinea pigs 1-7). Table 32 also shows that passive transfer was effected via serum.

6) All readings of palladium levels in sera of guinea pigs were zero.

7) Palladium levels in urine of guinea pigs showed significance only in pigs receiving 10 mg Pd - 1.5 mg alb complex (Table 33).

8) No significant levels of palladium were found in the liver, kidney or spleen of guinea pigs.

9) 14 of 18 mice in the first two groups (those receiving 1.5 and 0.15 mg alb) died by the fifth injection. This may have been due to the large amount of foreign protein per body weight or due to an allergic reaction (anaphylaxis) or a combination of both.

10) 7 of 9 mice in the third group (those receiving 10 mg Pd and 1.5 mg alb) died before footpad testing. It appears that the Pd-alb complex may inhibit any anaphylactic reaction since the survival rates are increased. However, the high concentration of palladium may in itself be toxic.

Only 2 of 9 mice in the fourth group died. Apparently, this concentration of palladium is better tolerated (1 mg Pd and 0.15 mg alb). None of the mice receiving 0.3 mg Pd and 0.05 mg alb died.

11) All surviving mice were footpad tested. No allergic induction was demonstrated by the footpad test; all tests were negative.

12) No significant palladium levels were found in the spleen, liver or kidney of mice.

CONCLUSIONS

1) There was no allergic induction to palladium by intravenous injection of Pd-alb complex into rabbits. This confirms the first Pd-alb experiment and also demonstrated an unexpected result. There was no development of immediate allergy to the albumin alone. The development of immediate allergy to albumin under these conditions would be expected, thus the rabbit appears not to be a good species.

2) Regarding the palladium levels in the sera and urine of rabbits injected intravenously with Pd-alb complex, the results are quite similar to the first Pd-alb complex experiment.

3) A delayed hypersensitivity (tuberculin type) was induced in guinea pigs by the subcutaneous injection of the Pd-alb complex. This hypersensitivity can be transferred to normal recipients by spleen cells but not by serum. Apparently, the palladium acts as a haptenic group on the albumin and prevents an immediate type allergy to the albumin from developing. Note that albumin alone injected subcutaneously into a guinea pig does cause an immediate allergy which can be transferred by serum. This guinea pig palladium or platinum/albumin complex system should provide a suitable model by which the immunological response to palladium or platinum can be studied.

4) No palladium levels were found in sera of guinea pigs subcutaneously injected with Pd-alb complex. This is similar to the first palladium-albumin complex experiment.

Significant palladium levels were found in the urine of guinea pigs subcutaneously injected with 10 mg Pd-1.5 mg alb complex only. Significant palladium levels were not found with lower Pd-alb complex concentrations. Again this is similar to the results of the first palladium-albumin complex experiment.

5) No allergic induction to palladium by intravenous injection of a Pd-alb complex in mice. Mice receiving high concentrations of albumin alone may have died due to anaphylaxis, but this is only a theoretical explanation and this result is not germaine to this project.

6) No significant palladium levels were found in the spleen, liver or kidney of the 3 species of animals when injected intravenously or in guinea pigs when injected subcutaneously, with a Pd-alb complex.

7) Finally, it appears that the guinea pig is the animal of choice for induction of an allergic state to palladium by the subcutaneous injection of a Pd-alb complex.

EXPERIMENT X

Pt-egg albumin complex in rabbits and guinea pigs

Rabbits and guinea pigs were grouped and subcutaneously injected with various concentrations of Pt-egg albumin complex 3 times a week for 3 weeks according to the following schedule:

a) Rabbits received three 1.0 ml subcutaneous injections per week for 3 weeks.

4 rabbits received 11.2 mg Pt/ml and 7 mg alb/ml
1 rabbit received 7 mg alb/ml

4 rabbits received 3 mg Pt/ml and 1.4 mg alb/ml
1 rabbit received 1.4 mg alb/ml

4 rabbits received 0.02 mg Pt/ml and 0.004 mg alb/ml
1 rabbit received 0.004 mg alb/ml

b) Guinea pigs received three 1.0 ml subcutaneous injections per week for three weeks.

4 guinea pigs received 11.2 mg Pt/ml and 7 mg alb/ml
1 guinea pig received 7 mg alb/ml

4 guinea pigs received 3 mg Pt/ml and 1.4 mg alb/ml
1 guinea pig received 1.4 mg alb/ml

4 guinea pigs received 0.02 mg Pt/ml and 0.004 mg alb/ml
1 guinea pig received 0.004 mg alb/ml

Results

1) All skin tests were negative in surviving animals.

2) Serum and urine levels of platinum in rabbits and guinea pigs are shown in Tables 34 - 37.
   a) In rabbits, significant levels of platinum were found only in the serum. Also, with the high concentrations (11.2 mg Pt), all rabbits died by one week (3rd injection). Fewer deaths occurred with the two lower platinum concentrations.
   b) In guinea pigs, significant levels of platinum were found in both serum and urine with all pigs receiving the high concentration (11.2 mg Pt) dying by the first week.

CONCLUSION

A dose of 11.2 mg Pt/ml subcutaneously 3 times appears to be toxic for both guinea pigs and rabbits; even the lower dose of 3 mg killed 60% of the animals. In the surviving animals, no allergic induction occurred.

EXPERIMENT XI

Rabbits and guinea pigs were grouped and subcutaneously injected with various concentration of Pt-egg albumin complex 3 times a week for 3 weeks according to the following schedule:

a) Rabbits received three 1.0 ml subcutaneous injections per week for 3 weeks

6 rabbits received 2.48 mg Pt and 0.02 mg alb/ml
1 rabbit received 0.02 mg alb/ml

6 rabbits received 0.25 mg Pt and 0.002 mg alb/ml
1 rabbit received 0.002 mg alb/ml

b) Guinea pigs received three 1.0 ml subcutaneous injections per week for 3 weeks

6 guinea pigs received 2.52 mg Pt and 0.02 mg alb/ml
1 guinea pig received 0.02 mg alb/ml

6 guinea pigs received 0.26 mg Pt and 0.002 mg alb/ml
1 guinea pig received 0.002 mg alb/ml

All animals were skin tested 14 days after the last subcutaneous injection.

Results

All skin tests were negative and no platinum was found in the sera or urine of either species.

CONCLUSION

Platinum alone or complexed to albumin at concentrations of 4.5 mg/ml or less would have minimal toxicity. However, platinum does not appear to induce an allergic state.

The guinea pig and the subcutaneous route of injection appear to be the best for allergic induction based upon experimental results using palladium complexed with egg albumin.

SECTION D

ALBUMIN COMPLEX

It has been established from Sections A, B and C that the guinea pig is the best of the three species (rabbit, guinea pig and mouse) used to induce an allergic state to platinum or palladium. The subcutaneous route is the best for exposure of the metal. Pt^{2+} or Pd^{2+} alone will not induce an allergic state. If, however, Pd^{2+} is complexed to egg albumin, the complex will sensitize (induce an allergic state in) the guinea pig. A Pt^{2+} - egg albumin complex will not induce an allergic state.

The effect of valences on the induction of the allergic state was studied by the following series of experiments.

EXPERIMENT XII

Guinea pigs were divided into ten groups and injected subcutaneously three times a week for four weeks with 1.0 ml of the solutions according to the following schedule:

Number of pigs	Solutions
5	$PtCl_4$ - 4.5 mg/ml
5	$PtCl_4$ - 1.5 mg/ml
5	$PtCl_4$ complexed to guinea pig albumin 4.5 mg/ml $PtCl_4$ and 0.070 mg/ml albumin
5	$PtCl_4$ complexed to guinea pig albumin 1.5 mg/ml $PtCl_4$ and 0.025 mg/ml albumin
5	$PtCl_4$ complexed to egg albumin 4.5 mg/ml $PtCl_4$ and 0.070 mg/ml albumin
5	$PtCl_4$ complexed to egg albumin 1.5 mg/ml $PtCl_4$ and 0.025 mg/ml albumin
4	Guinea pig albumin - 0.70 mg/ml
4	Guinea pig albumin - 0.025 mg/ml
4	Egg albumin - 0.70 mg/ml
4	Egg albumin - 0.025 mg/ml

Fourteen days after the last subcutaneous injection, all the guinea pigs were skin tested (intradermally) with 0.1 ml of the 10 solutions plus a buffer solution used as a diluent in the above 10 solutions. Skin reactions were checked at 30 min. and at 1, 9, 18, 24 and 48 hours.

Results

All skin test reactions were negative.

Comments

The failure of a platinum-albumin complex to induce an allergic reaction in guinea pigs while a similar palladium-albumin complex experiment has been successful in allergic induction, prompted the following observations:

1) The concentration of platinum per injection was lower (4.5 mg/ml) than that of the palladium (10 mg/ml). However, platinum concentrations higher than 4.5 mg/ml per injection were toxic. Also, delayed allergic response to palladium was induced with metals as low as 1.5 mg/ml per injection.

2) The albumin concentration was less in the present experiment (with platinum) than the concentration used in the palladium experiment. Thus the failure to induce an allergic response to platinum complexed to albumin and also to albumin itself suggests that a higher concentration of albumin may be necessary. Note: the reason a low albumin concentration was used was to determine the minimum albumin concentration needed to complex to the metal (platinum) for allergic induction.

EXPERIMENT XIII

To determine if the concentration of albumin is critical, a higher concentration was used here. Guinea pigs were divided into ten groups and injected subcutaneously three times a week for four weeks with 1.0 ml according to the following schedule:

Number of pigs	Solutions
5	$PtCl_4$ - 4.5 mg/ml
5	$PtCl_4$ - 1.5 mg/ml
5	$PtCl_4$ complexed to guinea pig albumin 4.5 mg/ml $PtCl_4$ and 7 mg/ml albumin
5	$PtCl_4$ complexed to guinea pig albumin 1.5 mg/ml $PtCl_4$ and 2.3 mg/ml albumin
5	$PtCl_4$ complexed to egg albumin 4.5 mg/ml $PtCl_4$ and 7 mg/ml albumin
5	$PtCl_4$ complexed to egg albumin 1.5 mg/ml $PtCl_4$ and 2.3 mg/ml albumin
4	Guinea pig albumin - 7 mg/ml
4	Guinea pig albumin - 2.3 mg/ml
4	Egg albumin - 7 mg/ml
4	Egg albumin - 2.3 mg/ml

Fourteen days after the last subcutaneous injection, all the guinea pigs were skin tested (intradermally) with 0.1 ml of the 10 solutions plus a buffer solution used as a diluent in the above 10 solutions. Skin reactions were checked at 30 min. and at 1, 9, 12, 24, and 48 hours.

Results

1) No positive skin tests in guinea pigs sensitized to $PtCl_4$ alone.

2) No positive skin tests in guinea pigs sensitized to $PtCl_4$ complexed to guinea pig albumin.

3) Guinea pigs sensitized to $PtCl_4$ complexed to egg albumin gave positive (delayed) skin tests only to guinea pig or egg albumin.

4) Guinea pigs sensitized to guinea pig albumin gave a positive delayed skin test reaction only to guinea pig albumin.

5) Guinea pigs sensitized to egg albumin gave a positive delayed skin test reaction to both guinea pig and egg albumin.

CONCLUSIONS

1) $PtCl_4$ injected subcutaneously (dose 1.4 - 5 mg/ml) does not induce an allergic response in guinea pigs.

2) $PtCl_4$ - guinea pig albumin or $PtCl_4$ -egg albumin complex injected subcutaneously does not induce an allergic response to platinum in guinea pigs. However, a delayed type allergy is induced to the albumin. This result differs from the palladium-egg albumin complex experiment. In that experiment a delayed type allergy to palladium was induced with no albumin allergy induction.

3) Thus far it appears that palladium complexed to egg albumin will induce an allergic state, while platinum complexed to egg albumin will not when injected subcutaneously into guinea pigs.

EXPERIMENT XIV

Guinea pigs were divided into six groups and were injected subcutaneously three times a week for three weeks with 1.0 ml according to the following schedule:

Number of pigs	Solutions
4	4.5 mg/ml $PdCl_4$ complexed to egg albumin
5	4.5 mg/ml $PdCl_4$ complexed to guinea pig albumin
5	3.8 mg/ml $PtCl_4$ complexed to egg albumin
4	3.8 mg/ml $PtCl_4$ complexed to guinea pig albumin
4	7.0 mg/ml egg albumin
5	7.0 mg/ml guinea pig albumin

Fourteen days after the last subcutaneous injection, all the guinea pigs were skin tested (intradermally) with a 0.1 ml of the 6 solutions plus a buffer used as a diluent in the above 6 solutions. Skin reactions were checked at ½, 1, 9, 18, 24 and 48 hours.

Results

1) Skin test reactions in guinea pigs injected with 4.5 $PdCl_4$ complexed to egg albumin. See Table 18.

2) Skin test reactions in guinea pigs injected with 4.5 $PdCl_4$ complexed to

guinea pig albumin. See Table 39.

3) Skin test reactions in guinea pigs injected with $PtCl_4$ complexed to egg albumin. All sites negative.

4) Skin test reactions in guinea pigs injected with $PtCl_4$ complexed to guinea pig albumin. All sites negative.

5) Skin test reactions in guinea pigs injected with egg albumin. Slight reaction in 2 of 4 pigs when skin tested with egg albumin or the palladium-egg albumin or the palladium-guinea pig albumin complex.

6) Skin test reactions in guinea pigs injected with guinea pig albumin. All sites negative.

7) Passive transfer - Skin test reaction in guinea pigs injected with spleen cells from palladium-egg albumin complex sensitive pigs. See Table 40.

8) Passive transfer - Skin test reactions in guinea pigs injected with spleen cells from palladium-guinea pig albumin complex sensitive pigs. See Table 41.

CONCLUSION AND COMMENT

1) Guinea pigs sensitized to palladium-egg albumin complex (4.5 mg/ml Pd and 7.0 mg/ml egg albumin) showed a delayed allergy when skin tested with palladium-egg albumin or palladium-guinea pig albumin complexes. No allergy developed to egg albumin or guinea pig albumin alone. It appears that in the palladium-egg albumin complex the palladium acts as an haptene, thus determing the antigenicity of the complex.

   Confirmation of the induction of delayed allergy to palladium-egg albumin complex was obtained via passive transfer.

   Spleen cells from palladium-egg albumin sensitive pigs, when injected intraperitoneally into normal recipients will confer a delayed type allergy.

2) Guinea pigs sensitized to palladium-guinea pig albumin complex (4.5 mg/ml Pd and 7.0 mg/ml guinea pig albumin) showed a delayed type allergy when skin tested with palladium-guinea pig albumin or palladium-egg albumin complexes.

   Passive transfer was also effected by the intraperitoneal injection into normal recipients spleen cells from guinea pigs sensitized to palladium-guinea pig albumin complex.

3) Guinea pigs injected with platinum-egg albumin or platinum-guinea pig albumin complexes (3.8 mg/ml platinum and 7.0 mg/ml of egg or guinea pig albumin) failed to induce an allergic response.

It thus appears that platinum alone or in the complexed state (complexed with egg or guinea pig albumin) will not induce any type of allergy. However, palladium when complexed to egg or guinea pig albumin will induce a delayed allergy which

can be transferred to normal recipients via spleen cells from sensitive donors.

Two additional experiments were designed to determine the minimal amount of palladium (in the complexed state) needed to induce the allergy.

EXPERIMENTS XV AND XVI

Guinea pigs were separated into 8 groups and injected subcutaneously three times a week for three weeks with 1.0 ml of various concentrations of palladium-albumin complex according to the following schedule:

Number of pigs	Solutions
7	2.0 mg/ml Pd complexed to 3.5 mg/ml guinea pig albumin
7	2.0 mg/ml Pd complexed to 3.5 mg/ml egg albumin
7	1.0 mg/ml Pd complexed to 1.7 mg/ml guinea pig albumin
7	1.0 mg/ml Pd complexed to 1.7 mg/ml egg albumin
7	0.80 mg/ml Pd complexed to 1.4 mg/ml guinea pig albumin
7	0.80 mg/ml Pd complexed to 1.4 mg/ml egg albumin
7	0.22 mg/ml Pd complexed to 0.75 mg/ml guinea pig albumin
7	0.22 mg/ml Pd complexed to 0.75 mg/ml egg albumin

Seven days after the last subcutaneous injection all guinea pigs were skin tested.

Results

All skin tests were negative.

CONCLUSIONS

Palladium concentrations of 2.0 mg/ml or less fail to induce an allergic state.

EXPERIMENT XVII

Guinea pigs were separated into 8 groups and injected subcutaneously three times a week for 3 weeks with 1.0 ml of various concentrations of palladium-albumin complex solution according to the following schedule:

Number of pigs	Solutions
7	3.7 mg/ml Pd complexed to 5.75 mg/ml egg albumin
7	3.2 mg/ml Pd complexed to 5.0 mg/ml egg albumin
7	2.7 mg/ml Pd complexed to 4.2 mg/ml egg albumin
7	3.7 mg/ml Pd complexed to 5.75 mg/ml guinea pig albumin
7	3.2 mg/ml Pd complexed to 5.2 mg/ml guinea pig albumin
7	2.7 mg/ml Pd complexed to 4.2 mg/ml guinea pig albumin
4	5.75 mg/ml egg albumin
4	5.75 mg/ml guinea pig albumin

Ten days after the last subcutaneous injection all guinea pigs were skin tested intradermally with 0.1 ml of each of the palladium-albumin (egg or guinea pig)

solutions plus buffer solution containing the three concentrations of palladium (without albumin), buffer solution containing egg or guinea pig albumin, and buffer alone for a total of 12 skin test solutions.

Results

Only guinea pigs injected with 3.7 mg/ml palladium complexed to 5.75 mg/ml egg albumin gave a positive skin test when tested with 0.1 ml of the 3.7 mg/ml Pd complexed to 5.75 mg/ml egg albumin. Table 42 lists the individual reaction areas. Passive transfer of the allergy was effected in four normal guinea pigs by intradermally injecting 5×10^8 spleen cells from 4 of the 6 sensitized guinea pigs. Table 43 lists the reaction areas in the recipient pigs. When skin tested 24 hr after receiving sensitized spleen cells.

CONCLUSIONS

A palladium concentration of 3.7 mg/ml appears to be the minimum concentration of palladium that will induce an allergic response when the palladium is complexed to egg albumin. If guinea pig albumin is used no allergic induction occurs.

EXPERIMENT XVIII

Guinea pigs were separated into 6 groups and injected subcutaneously three times a week for three weeks with 1.0 ml of various concentrations of a palladium-albumin complex solution according to the following schedule:

Number of pigs	Solutions
6	5.0 mg/ml Pd complexed to 7.0 mg/ml egg albumin
6	3.75 mg/ml Pd complexed to 7.0 mg/ml egg albumin
6	5.0 mg/ml Pd complexed to 7.0 mg/ml guinea pig albumin
6	3.75 mg/ml Pd complexed to 7.0 mg/ml egg albumin
2	7.0 mg/ml egg albumin
2	7.0 mg/ml guinea pig albumin

Ten days after the last subcutaneous injection all guinea pigs were skin tested intradermally with 0.1 ml of each of the palladium-albumin (egg or guinea pig) solutions plus buffer solution containing the 2 concentrations of palladium (without albumin), buffer solution containing egg or guinea pig albumin, and buffer alone for a total of 9 skin test solutions.

Results

Only guinea pigs injected with palladium complexed to egg albumin showed reactions upon skin testing. The only solutions used for skin testing that produced a positive reaction were 5.0 mg and 3.75 mg palladium complexed to egg albumin. These reaction areas are liuted in Table 44. Passive transfer of the sensitivity was successful and the skin test reactions in recipient guinea pigs are listed in Table 45.

CONCLUSIONS

1) Delayed allergy is induced with palladium complexed to egg albumin, minimum concentration 3.7 mg/ml.

2) Palladium concentrations higher than 5.0 mg/ml complexed to guinea pig albumin will induce a delayed allergy.

REFERENCES

1. Freedman, S.O. and J. Krupey. Respiratory allergy caused by platinum salts. J. Allergy 43:235. (1969).

2. Levene, G.M. and C. D. Calnan. Platinum hypersensitivity: treatment by specific hyposensitization. Clin. Allergy. 1:75. (1971).

3. Roberts, A. Platinosis - a five year study of the effects of soluble platinum salts on employees in a platinum laboratory and refinery. Arch. Ind. Hyg. 4:549. (1951).

4. Hunter, D. The diseases of occupations. The English University Press. LTD. London, Fourth Ed. p. 480. (1969).

5. Parrot, J. L., R. Herbert, A. Saindelle, and F. Ruff. Platinum and platinosis. Arch. Environ. Health 19:686. (1969).

6. Levene, G.M. Platinum sensitivity. Br. J. Derm. 85:590. (1971).

7. Saindelle, A. and F. Ruff. Histamine release by sodium chloroplatinate. Br. J. Pharmac. 35:313. (1969).

8. Hunter, D., R. Milton and K. M. A. Perry. Asthma caused by the complex salt of platinum. Brit. J. Indust. Med. 2:92. (1945).

TABLE I. Pd Levels at Various Times in Urine of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdCl_4$

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml	32 mg/ml
0.5	1	0.0000	0.0022	0.0019	0.1055	*	*
	2	0.0000	0.0101	*	*	*	*
	3	0.0000	0.0175	*	*	*	*
	4	0.0000	*	*	*	*	*
	5	0.0000	0.0570	*	*	*	*
0.1	6	*	*	*	*	*	*
	7	0.0000	0.0071	*	*	*	*
	8	0.0001	0.0015	0.0024	0.0108	0.0090	*
	9	0.0001	0.0015	0.0052	0.0104	0.0143	*
	10	0.0001	0.0073	0.0017	0.0097	*	*
0.05	11	0.0000	0.0019	0.0000	0.0008	0.0090	*
	12	0.0000	0.0069	0.0001	0.0029	*	*
	13	0.0006	0.0017	0.0004	0.0003	0.0100	0.0002
	14	0.0000	0.0059	0.0057	0.0049	0.0062	*
	15	*	*	*	*	*	*

*No results due to death of animal.

TABLE 2. Pd Levels at Various Times in Serum of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdSO_4$.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	9 mg/ml	16 mg/ml	23 mg/ml	30 mg/ml
0.5	1	0.0005	0.0000	0.0000	0.0002	*
	2	0.0004	*	*	*	*
	3	0.0005	*	*	*	*
	4	0.0004	*	*	*	*
	5	0.0001	*	*	*	*
0.1	6	*	*	*	*	*
	7	0.0001	0.0000	*	*	*
	8	0.0000	0.0000	0.0000	0.0001	*
	9	0.0001	0.0000	0.0002	0.0001	*
	10	0.0000	0.0002	0.0000	*	*
0.05	11	0.0002	0.0000	0.0000	0.0000	*
	12	0.0000	0.0002	0.0000	*	*
	13	0.0002	0.0000	0.0000	0.0000	0.0000
	14	0.0008	0.0000	0.0000	0.0007	0.0000
	15	*	*	*	*	*

*No results due to death of animal.

TABLE 3. Pd Levels at Various Times in Urine of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdSO_4$

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml	32 mg/ml
0.5	1	0.0000	0.0065	0.0020	0.0033	0.0010	0.0005
	2	0.0000	0.0026	0.0045	0.0000	*	*
	3	0.0009	0.0023	0.0000	0.0050	0.0010	0.0001
	4	*	*	*	*	*	*
	5	0.0000	0.0000	*	*	*	*
	6	0.0000	0.0042	0.0010	0.0060	0.0020	0.0001
0.1	7	0.0000	0.0000	0.0005	0.0009	0.0001	0.0000
	8	0.0002	0.0050	0.0006	0.0011	0.0001	0.0000
	9	0.0000	0.0004	0.0003	0.0010	0.0000	*
	10	0.0006	0.0006	0.0008	0.0005	0.0001	0.0000
0.05	11	0.0000	0.0013	0.0003	0.0005	0.0001	0.0000
	12	0.0001	0.0022	0.0001	0.0005	0.0001	0.0000
	13	0.0000	0.0018	0.0014	0.0012	0.0001	0.0000
	14	0.0000	0.0009	0.0004	0.0005	*	*
	15	*	*	*	*	*	*

*No results due to death of animal.

TABLE 4. Pd Levels at Various Times in Serum of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdSO_4$

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	9 mg/ml	16 mg/ml	23 mg/ml	30 mg/ml
0.5	1	0.0000	0.0000	0.0002	0.0003	0.0002
	2	0.0000	0.0004	*	*	*
	3	0.0005	0.0001	0.0000	0.0000	0.0001
	4	*	*	*	*	*
	5	0.0000	0.0008	*	*	*
	6	0.0003	0.0001	0.0002	0.0002	0.0000
0.1	7	0.0000	0.0000	0.0000	0.0000	0.0000
	8	0.0002	0.0000	0.0002	0.0000	0.0000
	9	0.0000	0.0000	0.0000	0.0001	0.0000
	10	0.0006	0.0000	0.0006	0.0007	0.0000
0.05	11	0.0000	0.0001	0.0000	0.0001	0.0000
	12	0.0003	0.0000	0.0000	0.0001	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0001
	14	0.0000	0.0000	0.0002	0.0001	*
	15	*	*	*	*	*

*No results due to death of animal.

TABLE 5. Pt Levels at Various Times in Urine of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	7 mg/ml	14 mg/ml	21 mg/ml	35 mg/ml
0.35	1	0.0000	0.0020	0.0035	0.0015	0.0013
	2	0.0000	0.0030	0.0008	0.0005	0.0000
	3	0.0000	0.0010	0.0000	0.0025	0.0007
	4	0.0000	0.0005	0.0006	0.0040	0.0000
	5	0.0000	0.0000	0.0000	0.0025	0.0000
0.1	6	0.0010	0.0000	0.0000	0.0000	*
	7	0.0005	0.0000	0.0000	0.0005	0.0000
	8	0.0005	0.0020	0.0000	0.0010	0.0000
	9	0.0005	0.0015	0.0006	0.0005	0.0000
	10	0.0010	0.0000	0.0000	0.0000	0.0000
0.05	11	0.0005	0.0010	0.0012	0.0010	0.0000
	12	0.0005	0.0005	0.0000	0.0000	0.0000
	13	0.0000	0.0015	0.0006	0.0005	0.0000
	14	0.0000	0.0005	0.0000	0.0000	0.0000
	15	0.0000	0.0000	0.0000	0.0005	0.0000

*No results due to death of animal.

TABLE 6. Pi Levels at Various Times in Serum of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PiSO_4$.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	9 mg/ml	16 mg/ml	21 mg/ml	35 mg/ml
0.35	1	0.0000	0.0004	0.0004	0.0006	0.0000
	2	0.0000	0.0004	0.0000	0.0006	0.0000
	3	0.0000	0.0000	0.0000	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0006	0.0000
	5	0.0000	0.0000	0.0000	0.0006	0.0000
0.1	6	0.0005	0.0004	0.0000	0.0003	*
	7	0.0000	0.0000	0.0000	0.0006	0.0000
	8	0.0000	0.0004	0.0000	0.0000	0.0000
	9	0.0000	0.0004	0.0000	0.0006	0.0000
	10	0.0000	0.0004	0.0000	0.0000	0.0000
0.05	11	0.0000	0.0000	0.0004	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0004	0.0000	0.0006	0.0000
	14	0.0005	0.0000	0.0000	0.0000	0.0000
	15	0.0000	0.0004	0.0000	0.0006	0.0000

*No results due to death of animal.

TABLE 7. Pt Levels at Various Times in Urine of Rabbits Injected S.C. 1 Times a Week for 4 Weeks with Various Concentrations of $PtCO_4$.

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Rabbit	0 mg/ml	7 mg/ml	14 mg/ml	21 mg/ml	28 mg/ml
0.25	1	0.0000	0.0015	0.0006	0.0010	0.0013
	2	0.0000	0.0015	0.0006	0.0010	0.0000
	3	0.0000	0.0005	0.0006	*	*
	4	0.0000	0.0005	0.0012	0.0000	0.0000
	5	0.0000	0.0020	0.0006	0.0000	0.0000
0.1	6	0.0000	0.0005	0.0006	0.0005	*
	7	0.0010	0.0015	0.0006	0.0000	0.0013
	8	0.0000	0.0010	0.0006	0.0000	0.0000
	9	0.0000	0.0005	0.0000	0.0010	0.0000
	10	0.0000	0.0010	0.0006	0.0000	0.0000
0.05	11	0.0005	0.0005	0.0000	0.0000	0.0000
	12	0.0000	0.0005	0.0000	0.0005	0.0000
	13	0.0000	0.0005	0.0006	0.0000	0.0000
	14	0.0000	0.0010	0.0000	0.0005	0.0013
	15	0.0000	0.0010	0.0000	0.0000	*

*No results due to death of animal.

TABLE 8. Pt Levels at Various Times in Serum of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	9 mg/ml	16 mg/ml	21 mg/ml	35 mg/ml
0.35	1	0.0000	0.0004	0.0000	0.0006	0.0000
	2	0.0000	0.0004	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	*	*
	4	0.0000	0.0000	0.0000	0.0000	0.0000
	5	0.0005	0.0004	0.0000	0.0000	0.0000
0.1	6	0.0000	0.0004	0.0000	0.0006	*
	7	0.0000	0.0004	0.0000	0.0000	0.0000
	8	0.0000	0.0000	0.0000	0.0000	0.0000
	9	0.0000	0.0004	0.0000	0.0000	0.0000
	10	0.0000	0.0004	0.0000	0.0000	0.0000
0.05	11	0.0000	0.0000	0.0000	0.0006	0.0000
	12	0.0000	0.0000	0.0000	0.0006	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0000	0.0000	0.0000	0.0000	0.0000
	15	0.0005	0.0004	0.0000	*	*

*No results due to death of animal.

TABLE 9. Average concentration of $PtSO_4$ in splenic tissue of guinea pigs, rabbits and mice injected with various concentrations of $PtSO_4$ 3 times a week for 4 weeks.

	Guinea Pigs		Rabbits		Mice	
Inj. Dose mg/ml	no. of animals	avg. $PtSO_4$ in spleen mg/gm	no. of animals	avg. $PtSO_4$ in spleen mg/gm	no. of animals	avg. $PtSO_4$ in spleen mg/gm
0.15	5	0.0503	5	0.0164	4	0.1414
0.10	5	0.0296	5	0	5	0.2865
0.05	5	0.0288	5	0.0118	5	0

TABLE 10. Levels of $PdSO_4$ and $PtSO_4$ in the urine of rabbits and guinea pigs.

Inj. dose mg/ml	Rabbits: maximum levels of metal in urine mg/ml $PdSO_4$	Rabbits: maximum levels of metal in urine mg/ml $PtSO_4$	Guinea Pigs: maximum levels of metal in urine mg/ml $PdSO_4$	Guinea Pigs: maximum levels of metal in urine mg/ml $PtSO_4$
0.35	0.0047	0.0012	0.0210	0.0012
0.10	0.0015	0.0009	0.0117	0.0007
0.05	0.0015	0.0007	0.0084	0.0007

TABLE II. Pt Levels at Various Times in Serum of Rabbit Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$ + NH_4Cl.

(Significant to 0.0005 mg/ml)

Conc. Inj.	Rabbit	Day of Experiment 0 mg/ml	5 mg/ml	12 mg/ml	19 mg/ml	26 mg/ml
0.1 mg $PtSO_4$/ml	1	0.0000	0.0000	0.0000	0.0008	0.0010
and	2	0.0000	0.0000	0.0000	0.0000	0.0000
0.2 g NH_4Cl/ml	3	0.0000	0.0000	0.0000	0.0008	0.0000
0.1 mg $PtSO_4$/ml	4	0.0000	0.0010	0.0000	0.0000	0.0000
and	5	0.0000	0.0010	0.0007	0.0000	0.0010
0.07 g NH_4Cl/ml	6	0.0000	0.0010	0.0000	0.0000	0.0000
0.05 mg $PtSO_4$/ml	7	0.0008	0.0000	0.0007	*	*
and	8	0.0000	0.0010	0.0007	0.0008	0.0000
0.03 g NH_4Cl/ml	9	0.0008	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.

TABLE 12. Pt Levels at Various Times in Urine of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$ + NH_4Cl.

(Significant to 0.001 mg/ml)

Conc. Inj.	Rabbit	Day of Experiment 0 mg/ml	7 mg/ml	13 mg/ml	20 mg/ml	27 mg/ml
0.5 mg $PtSO_4$/ml	1	0.0000	0.0000	0.0010	0.0004	0.0004
and	2	0.0000	0.0000	0.0005	0.0000	0.0000
0.4 g NH_4Cl/ml	3	0.0000	0.0000	0.0000	0.0000	*
0.1 mg $PtSO_4$/ml	4	0.0005	0.0000	0.0000	0.0000	0.0011
and	5	0.0000	0.0005	0.0015	0.0004	0.0004
0.07 g NH_4Cl/ml	6	0.0000	0.0000	0.0010	0.0004	0.0004
0.05 mg $PtSO_4$/ml	7	0.0000	0.0005	0.0005	*	*
and	8	0.0000	0.0000	0.0000	0.0000	0.0000
0.03 g NH_4Cl/ml	9	0.0000	0.000	0.0000	0.0000	0.0007

*No result due to death of animal.

TABLE 15. Pt Levels at Various Times in Serum of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$ + NH_4Cl.

(Significant to 0.0005 mg/ml)

Conc. Inj.	Guinea Pig	Day of Experiment 0 mg/ml	5 mg/ml	12 mg/ml	19 mg/ml	26 mg/ml
0.3 mg $PtSO_4$ /ml	1	0.0008	0.0010	*	*	*
and	2	0.0000	*	*	*	*
0.2 g NH_4Cl/ml	3	0.0000	*	*	*	*
0.1 mg $PtSO_4$/ml	4	0.0000	0.0010	0.0000	0.0008	0.0020
and	5	0.0000	0.0000	*	*	*
0.07 g NH_4Cl/ml	7	0.0000	0.0010	0.0000	0.0008	0.0010
0.05 mg $PtSO_4$ /ml	9	0.0000	0.0000	0.0007	0.0000	0.0010
and	10	0.0000	0.0000	0.0000	0.0000	0.0010
0.03 g NH_4Cl/ml	11	0.0000	0.0010	0.0000	0.0000	0.0000

*No results due to death of animal.

TABLE 14. Pt Levels at Various Times in Urine of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PtSO_4$ + NH_4Cl.

(Significant to 0.001 mg/ml)

Conc. Inj.	Guinea Pig	0 mg/ml	7 mg/ml	13 mg/ml	20 mg/ml	27 mg/ml
0.1 mg $PtSO_4$ /ml	1	0.0000	*	*	*	*
and	2	0.0005	*	*	*	*
0.2 g NH_4Cl/ml	3	0.0000	*	*	*	*
0.1 mg $PtSO_4$ /ml	4	0.0005	0.0000	0.0005	0.0000	0.0000
and	5	0.0000	*	*	*	*
0.07 g NH_4Cl/ml	7	0.0005	0.0000	0.0000	0.0004	0.0004
0.05 mg $PtSO_4$ /ml	9	**	0.0000	0.0005	0.0000	0.0004
and	10	0.0000	0.0000	0.0000	0.0000	0.0000
0.03 g NH_4Cl/ml	11	0.0000	0.0000	0.0000	0.0004	0.0000

*No results due to death of animal
**No urine collected.

TABLE 15. Pd Levels at Various Times in Urine of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdBO_4$.

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml
0.3	1	0.0000	0.0006	0.0000	0.0006	0.0009
	2	0.0000	0.0009	0.0000	0.0007	0.0004
	3	0.0030	0.0003	0.0003	0.0003	0.0006
	4	0.0000	0.0005	0.0000	0.0005	0.0005
	5	0.0000	0.0006	0.0000	0.0009	0.0007
0.1	6	0.0000	0.0005	0.0000	0.0000	0.0005
	7	0.0000	0.0002	0.0000	*	*
	8	0.0000	0.0002	0.0000	*	*
	9	0.0000	0.0001	0.0000	0.0000	0.0004
	10	0.0000	0.0001	0.0000	*	*
0.05	11	0.0000	0.0000	*	*	*
	12	0.0000	0.0003	0.0000	0.0005	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0000	0.0000	0.0000	0.0001	0.0001
	15	0.0000	0.0000	0.0000	0.0000	0.0001

*No results due to death of animal.

TABLE 16. Pd Levels at Various Times in Serum of Guinea Pigs Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdBO_4$.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	2 mg/ml	9 mg/ml	16 mg/ml	23 mg/ml
0.3	1	0.0000	0.0000	0.0000	0.0000	0.0000
	2	0.0000	0.0000	0.0002	0.0000	0.0000
	3	0.0000	0.0000	0.0002	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0000	0.0000
	5	0.0000	0.0000	0.0000	0.0000	0.0000
0.1	6	0.0000	0.0000	0.0000	0.0000	0.0000
	7	0.0000	0.0000	0.0000	0.0000	*
	8	0.0000	0.0000	0.0000	0.0000	*
	9	0.0000	0.0000	0.0002	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000	*
0.05	11	0.0000	0.0000	*	*	*
	12	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0000	0.0000	0.0002	0.0000	0.0000
	15	***	0.0000	0.0002	0.0000	0.0000

*No results due to death of animal.

***No blood was obtained.

TABLE 17. Pd Levels at Various Times in Urine of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdSO_4$.

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml
0.5	1	0.0000	0.0000	0.0000	0.0000	*
	2	0.0000	0.0000	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0000	0.0000
	5	0.0000	0.0000	0.0000	0.0002	0.0000
0.1	6	0.0000	0.0000	0.0000	0.0000	0.0000
	7	0.0000	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0002	0.0002	0.0002	0.0000
	9	0.0000	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000	0.0000
0.05	11	0.0000	0.0000	0.0000	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0000	0.0000	0.0000	0.0000	0.0000
	15	*	*	*	*	*

*No results due to death of animal.

TABLE 18. Pd Levels at Various Times in Serum of Rabbits Injected S.C. 3 Times a Week for 4 Weeks with Various Concentrations of $PdSO_4$.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	2 mg/ml	9 mg/ml	16 mg/ml	23 mg/ml
0.5	1	0.0000	0.0000	0.0000	0.0000	0.0000
	2	0.0000	0.0000	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0002	0.0000
	5	0.0000	0.0000	0.0000	0.0000	0.0000
0.1	6	0.0000	0.0000	0.0000	0.0000	0.0000
	7	0.0000	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0000	0.0000	0.0000	0.0000
	9	0.0000	0.0000	0.0002	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000	0.0000
0.05	11	0.0000	0.0000	0.0000	0.0000	0.0003
	12	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0002
	14	0.0000	0.0000	0.0000	0.0000	0.0000
	15	*	*	*	*	*

*No results due to death of animal.

TABLE 19. Average Maximum Levels of Metal in the Urine of Animals in mg/ml. (Significant to 0.005 mg/ml)

Injection Dose mg/ml	RABBITS Injected with				GUINEA PIGS Injected with			
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.35	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.30	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.25	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE 20. Average Maximum Levels of Metal in the Serum of Animals in mg/ml (Significant to 0.0005 mg/ml)

Injection Dose mg/kg	RABBITS Injected with: $Pd(SO_3)_2(NH_4)_2Cl$	$Pd(SO_3)_2$	$Pt(SO_3)_2(NH_4)_2Cl$	$Pt(SO_3)_2$	GUINEA PIGS Injected with: $Pd(SO_3)_2(NH_4)_2Cl$	$Pd(SO_3)_2$	$Pt(SO_3)_2(NH_4)_2Cl$	$Pt(SO_3)_2$
0.35	0.0001	0.0000	0.0005	0.0002	All died	0.0000	0.0000	0.0004
0.10	0.0000	0.0000	0.0003	0.0005	0.0000	0.0000	0.0005	0.0003
0.05	0.0000	0.0000	0.0002	0.0004	0.0000	0.0000	0.0007	0.0002

TABLE 21. Pt Levels at Various Times in Urine of Rabbits Injected I.V. 3 Times a Week for 3 Weeks with Various Concentrations of $PtBO_4$:

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml
0.3	1	0.0004	0.0005	0.0006	0.0007
	2	0.0000	0.0015	0.0000	0.0000
	3	**	0.0000	0.0000	0.0000
	4	0.0000	0.0015	0.0006	0.0000
	5	0.0000	0.0000	0.0000	0.0007
0.1	6	0.0000	0.0000	0.0000	0.0000
	7	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0005	0.0000	0.0013
	9	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000
0.05	11	0.0000	0.0005	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000
	14	*	*	*	*
	15	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.

**No urine.

TABLE 22. Pt Levels at Various Times in Serum of Rabbits Injected I.V. 3 Times a Week for 3 Weeks with Various Concentrations of $PtSO_4$.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	2 mg/ml	9 mg/ml	16 mg/ml
0.5	1	0.0000	0.0000	0.0000	0.0010
	2	0.0000	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0010
	5	0.0000	0.0000	0.0000	0.0010
0.1	6	0.0000	0.0000	0.0000	0.0000
	7	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0000	0.0000	0.0000
	9	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0000	0.0020	0.0000
0.05	11	0.0000	0.0000	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000
	14	*	*	*	*
	15	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.

TABLE 11. Pt Levels at Various Times in Urine of Rabbits Exposed Once a Week for 5 Weeks to Various Concentrations of $PtSO_4$ Paste.

(Significant to 0.001 mg/ml)

Conc. %	Rabbit	Day of Experiment 0 mg/ml	3 mg/ml	10 mg/ml	17 mg/ml	24 mg/ml	31 mg/ml
0.05	1	0.0000	0.0000	0.0004	0.0015	0.0011	0.0007
	2	0.0006	0.0006	0.0004	0.0015	0.0011	0.0007
	3	0.0011	0.0000	0.0000	0.0015	0.0000	0.0000
	13	0.0006	0.0000	0.0004	0.0006	0.0000	0.0000
	5	0.0017	0.0000	0.0009	0.0010	0.0000	0.0007
	6	0.0017	0.0011	0.0009	0.0010	0.0000	0.0013
	14	0.0006	0.0011	0.0013	0.0015	0.0000	0.0027
0.10	7	0.0006	0.0000	0.0000	0.0006	0.0000	0.0000
	8	0.0000	0.0006	0.0000	0.0000	0.0000	0.0007
	9	0.0000	0.0000	0.0000	0.0181*	0.0000	0.0007
	16	0.0011	0.0000	0.0000	0.0005	0.0000	0.0000
	11	0.0000	0.0000	0.0009	0.0000	0.0006	0.0020
	12	0.0011	0.0006	0.0004	0.0000	0.0006	0.0040
	15	0.0000	0.0000	0.0004	0.0015	0.0000	0.0000

*Rabbit removed patch and $PtSO_4$ fell into urine collection pan.

TABLE 44. Pt Levels at Various Times in Serum of Rabbits Exposed Once a Week for 5 Weeks to Various Concentrations of $PtSO_4$ Paste.

(Significant to 0.0005 mg/ml)

Conc. %	Rabbit	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml	32 mg/ml
0.25	1	0.0005	0.0000	0.0000	0.0006	0.0000	0.0000
	2	0.0000	0.0005	0.0005	0.0000	0.0000	0.0000
	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000
0.10	7	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000
	11	0.0000	0.0005	0.0000	0.0000	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	15	0.0005	0.0005	0.0000	0.0000	0.0000	0.0000

TABLE 25. Pt Levels at Various Times in Urine of Guinea Pigs Exposed Once a Week for 5 Weeks to Various Concentrations of $PtSO_4$ Paste.

(Significant to 0.001 mg/ml)

Conc. %	Guinea Pig	Day of Experiment 0 mg/ml	3 mg/ml	10 mg/ml	17 mg/ml	24 mg/ml	31 mg/ml
0.25	1	0.0000	0.0000	0.0009	0.0000	0.0006	0.0000
	2	0.0005	0.0005	0.0437**	0.0040	*	*
	3	0.0005	0.0005	0.0027	0.0035	0.0000	0.0020
	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0007
	5	0.0000	0.0000	*	*	*	*
	6	0.0000	0.0000	0.0009	0.0000	0.0000	0.0040
	7	0.0000	0.0000	0.0004	0.0000	0.0017	0.0151**
	8	0.0011	0.0005	0.0022	0.0010	0.0017	0.0007
0.10	9	0.0006	0.0000	0.0000	0.0010	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000	0.0006	***
	11	0.0000	0.0000	0.0009	*	*	*
	12	0.0006	0.0000	0.0004	0.0005	0.0000	0.0007
	13	0.0006	0.0006	0.0004	0.0000	0.0000	0.0000
	14	0.0011	0.0000	0.0004	0.0010	0.0000	0.0020
	15	0.0006	0.0000	0.0007	0.0010	0.0000	0.0007

*No results due to death of animal.
**Unusual high reading due to the pigs removal of the patch and its falling into the urine collection pan.
***No urine collected.

TABLE 26. Pt Levels at Various Times in Serum of Guinea Pigs Exposed Once a Week for 5 Weeks to Various Concentrations of $PtCl_4$ Paste.

(Significant to 0.0005 mg/ml)

Conc. %	Guinea Pig	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml	25 mg/ml	32 mg/ml
0.25	1	0.0000	0.0000	0.0005	0.0005	0.0000	0.0000
	2	0.0000	0.0005	0.0000	0.0000	*	*
	3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5	0.0005	0.0000	0.0000	0.0000	0.0000	*
	6	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000
	7	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000
	8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
0.10	9	0.0005	0.0000	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	11	0.0000	0.0000	0.0000	*	*	*
	12	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	13	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	14	0.0005	0.0000	0.0005	0.0000	0.0000	*
	15	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.

TABLE 27. Pd Levels at Various Times in Urine of Guinea Pigs Injected I.V. 3 Times a Week for 3 Weeks with Various Concentrations of Pd-Albumin Complex.*

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	4 mg/ml	11** mg/ml	18 mg/ml
10.0	1	0.0000	0.0000	0.0029	0.0021
	2	0.0000	0.0005	**	**
	3	0.0000	**	**	**
	4	0.0000	0.0023	0.0020	0.0025
	5	0.0000	**	**	**
1.0	6	0.0000	0.0000	0.0000	0.0001
	7	0.0000	0.0001	0.0001	0.0005
	8	0.0000	0.0002	0.0000	0.0006
	9	0.0000	0.0005	0.0001	0.0002
	10	0.0000	0.0001	0.0000	0.0001
0.1	11	0.0000	0.0001	0.0001	0.0002
	12	0.0000	0.0002	0.0001	0.0002
	13	0.0000	0.0002	0.0001	0.0002
	14	0.0000	0.0001	0.0000	0.0000
	15	0.0000	0.0003	**	**

*After 4 injections animal received complex S.C.
**No Results due to death of animal.

TABLE 28. Pd Levels at Various Times in Urine of Rabbits Injected I.V. 3 Times a Week for 3 Weeks with Various Concentrations of Pd-Albumin Complex.

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	4 mg/ml	11 mg/ml	18 mg/ml
10.0	1	0.0000	0.0002	0.0001	0.0000
	2	0.0000	0.0003	0.0004	0.0002
	3	0.0000	0.0001	0.0006	0.0000
	4	0.0000	0.0004	0.0007	0.0004
	5	0.0000	*	*	*
1.0	6	0.0000	0.0001	0.0001	0.0001
	7	0.0000	0.0003	0.0001	0.0000
	8	0.0000	0.0001	0.0001	0.0002
	9	0.0000	0.0001	0.0002	0.0002
	10	0.0000	0.0000	0.0000	0.0001
0.3	11	0.0000	0.0001	0.0001	0.0004
	12	0.0000	0.0001	0.0001	0.0001
	13	0.0000	0.0001	0.0000	0.0001
	14	0.0000	0.0002	0.0002	0.0000
	15	0.0000	0.0001	0.0000	0.0001

*No results due to death of animal.

TABLE 29. Skin test reactions in guinea pigs in mm^2 (erythema and induration).

Guinea Pig Sensitive to	Hour	Skin tested with 0.1 ml intradermal injection of: 1.5 mg alb	0.15 mg alb	1.0 mg Pvt + 1.5 mg alb	1 mg Pvt + 0.15 mg alb	0.3 mg Pvt + 0.045 mg alb	Buffer
10 mg Pvt + 1.5 mg alb	0	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	24	0	0	151	30	9	0
	30	0	0	64	75	9	0
	48	0	0	49	25	9	0
10 mg Pvt + 1.5 mg alb	0	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	24	0	0	80	20	25	0
	30	0	0	56	9	16	0
	48	0	0	64	15	25	0
1 mg Pvt + 0.15 mg alb	0	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	24	0	0	24	20	25	0
	30	0	0	25	16	16	0
	48	0	0	20	15	9	0
1 mg Pvt + 0.15 mg alb	0	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	24	0	0	77	20	16	0
	30	0	0	77	20	20	0
	48	0	0	70	25	25	0
0.3 mg Pvt + 0.05 mg alb	0	0	0	0	0	0	0
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	24	0	0	180	35	12	0
	30	0	0	56	9	9	0
	48	0	0	64	9	9	0

TABLE 10. Rabbit urine palladium levels in mg/ml

(Significant to 0.001 mg/ml)

Injection	Pre-Injection	Day 7	Day 14	Day 21
10 mg Pd 1/5 mg alb	0.00006	0.0001	0.00016	0
10 mg Pd 1.5 mg alb	0	0.0006	0.00065	0.0003
1 mg Pd 0.15 mg alb	0	0.00007	0.00008	0
1 mg Pd 0.15 mg alb	0.00006	0	0	0
0.3 mg Pd 0.05 mg alb	0	0	0.00008	0

Note: All sera readings were zero.

TABLE II. Skin test reactions in guinea pigs in mm^2 (erythema and induration)

Guinea Pig Sensitive to	Hour	Skin tested with 0.1 ml intradermal injection of: 1.5 mg alb	0.15 mg alb	10 mg Pct 1.5 mg alb	1 mg Pct 0.15 mg alb	0.1 mg Pct 0.05 mg alb	Buffer
G.P. #1	½	121	132	–	–	–	0
	1	144	100	–	–	–	0
1.5 mg alb	2	180	120	–	–	–	0
	5.5	240	210	–	–	–	0
	24	0	0	–	–	–	0
	48	0	0	–	–	–	0
G.P. #2	½	121	100	–	–	–	0
	1	169	121	–	–	–	0
1.5 mg alb	2	196	130	–	–	–	0
	5.5	484	182	–	–	–	0
	24	0	0	–	–	–	0
	48	0	0	–	–	–	0
G.P. #3	½	120	120	–	–	–	0
	1	120	100	–	–	–	0
1.5 mg alb	2	255	120	–	–	–	0
	5.5	320	210	–	–	–	0
	24	0	0	–	–	–	0
	48	0	0	–	–	–	0
G.P. #4	½	143	0	–	–	–	0
	1	110	0	–	–	–	0
1.5 mg alb	2	195	0	–	–	–	0
	5.5	625	255	–	–	–	0
	24	0	0	–	–	–	0
	48	0	0	–	–	–	0

| | | |

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C. P. [illegible]	1	0	0	0	0	0	[illegible]
	1	0	0	0	[illegible]	0	0
1 mg Pot	2	0	0	0	0	0	0
0.35 mg	5.5	0	0	0	0	36	0
alb	26	0	0	121	12	25	0
	27	0	0	90	25	36	0
	48	0	0	72	9	9	0
C. P. [illegible]	1	0	0	0	0	0	0
	1	0	0	0	0	6	0
1 mg Pot	2	0	0	0	0	0	0
0.35 mg	5.5	0	0	0	4	25	0
alb	26	0	0	210	49	36	0
	27	0	0	196	72	36	0
	48	0	0	132	9	9	0
C. P. [illegible]	1	0	0	0	0	0	0
	1	0	0	0	0	0	0
0.3 mg Pot	2	0	0	0	0	0	0
0.05 mg alb	5.5	0	0	0	12	28	0
	26	0	0	42	16	49	0
	27	0	0	72	30	30	0
	48	0	0	16	9	9	0
C. P. [illegible]	1	0	0	0	0	0	0
	1	0	0	0	0	0	0
0.3 mg Pot	2	0	0	0	0	0	0
0.05 mg	5.5	0	0	0	0	0	0
alb	26	0	0	130	36	36	0
	27	0	?	144	25	9	0
	48	0	0	25	36	0	0

TABLE 32. Skin test reaction in mm^2 (erythema and induration) in normal guinea pigs receiving cells or sera from sensitized pigs and skin tested with 0.1 ml intradermally.

	Hour	1.5 mg alb	0.15 mg alb	1.5 mg alb 10 mg Pd	0.15 mg alb 1 mg Pd	0.885 mg alb 0.3 mg Pd	Buffer
G. P. 61	½	0	0	–	–	–	0
received	1	0	0	–	–	–	0
spleen	2	0	0	–	–	–	0
cells (10^7)	6	0	0	–	–	–	0
from alb	24	0	0	–	–	–	0
ser.	48	0	0	–	–	–	0
G.P. 62	½	0	0	0	0	0	0
received	1	0	0	0	0	0	0
spleen	2	0	0	0	0	0	0
cells (10^7)	6	0	0	50	35	4	0
from Pd-	24	0	0	64	35	12	0
alb. ser.	48	0	0	40	4	0	0
G.P. 65	½	0	0	0	0	0	0
received	1	0	0	0	0	0	0
spleen	2	0	0	0	0	0	0
cells (10^7)	6	0	0	25	9	9	0
from Pd-	24	0	0	56	6	4	0
alb ser.	48	0	0	25	0	0	0
G. P. 66	½	0	0	–	–	–	0
received	1	96	70	–	–	–	±
serum	2	100	70	–	–	–	0
from alb	6	0	0	–	–	–	0
ser.	24	0	0	–	–	–	0
	48	0	0	–	–	–	0

TABLE 11: Guinea pig urine palladium levels in mg/ml

(Significant to 0.001 mg/ml)

Injection	Guinea Pig	Pre-injection	Day 7	Day 14
10 mg Pd 1.5 mg alb complex	1	0	0.0029	0.0018
	2	0	0.0017	0.0022
1 mg Pd 0.15 mg alb complex	3	0.00006	0.0001	0.0005
	4	0	0.0004	0.0004
0.1 mg Pd 0.05 mg alb complex	5	0	0.00007	0.0003
	6	0	0.0001	0.0002

TABLE 14. Pt Levels at Various Times in Serum of Rabbits Injected S.C. 3 Times a Week for 3 Weeks with Various Concentrations of Pt-Albumin Complex.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	5 mg/ml	12 mg/ml	19 mg/ml
11.7	1	0.0000	0.0016	*	*
	2	0.0008	0.0016	*	*
	3	0.0008	0.0016	*	*
	4	0.0000	0.0008	*	*
2.6	5	0.0000	0.0000	0.0008	0.0000
	6	0.0000	0.0000	0.0000	0.0008
	7	0.0000	0.0008	*	*
	8	0.0000	0.0000	0.0000	0.0008
0.62	9	0.0000	0.0008	0.0000	0.0008
	10	0.0000	0.0000	0.0008	*
	11	0.0000	0.0000	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.

TABLE 35. Pt Levels at Various Times in Urine of Rabbits Injected S.C. 3 Times a Week for 3 Weeks with Various Concentrations of Pt-Albumin Complex.

(Significant to 0.001 mg/ml)

Conc. inj. mg/ml	Rabbit	Day of Experiment 0 mg/ml	7 mg/ml	14 mg/ml	19 mg/ml
11.7	1	0.0006	0.0009	*	*
	2	0.0006	0.0006	*	*
	3	0.0006	*	*	*
	4	0.0006	*	*	*
3.6	5	0.0006	0.0014	0.0009	0.0017
	6	0.0009	0.0009	0.0029	0.0011
	7	0.0006	0.0000	*	*
	8	0.0006	0.0003	0.0026	0.0023
0.02	9	0.0006	0.0009	0.0006	0.0009
	10	0.0003	0.0000	0.0009	*
	11	0.0006	0.0006	0.0011	0.0011
	12	0.0003	0.0009	0.0011	0.0009

*No results due to death of animal.

TABLE 16. Pt Levels at Various Times in Serum of Guinea Pigs Injected S.C. 3 Times a Week for 3 Weeks with Various Concentrations of Pt-Albumin Complex.

(Significant to 0.0005 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	5 mg/ml	12 mg/ml	19 mg/ml
11.7	1	0.0000	*	*	*
	2	**	*	*	*
	3	**	*	*	*
	4	0.0000	0.0024	*	*
2.6	5	0.0000	0.0008	0.0024	*
	6	0.0000	0.0008	0.0024	0.0024
	7	0.0000	0.0008	0.0016	0.0012
	8	0.0000	0.0008	*	*
0.03	9	0.0000	0.0000	0.0000	0.0000
	10	0.0000	0.0000	0.0000	0.0000
	11	0.0000	0.0000	0.0000	0.0000
	12	0.0000	0.0000	0.0000	0.0000

*No results due to death of animal.
**No serum.

TABLE 17. Pt Levels at Various Times in Urine of Guinea Pigs Injected S.C. 3 Times a Week for 3 Weeks with Various Concentrations of Pt-Albumin Complex.

(Significant to 0.001 mg/ml)

Conc. Inj. mg/ml	Guinea Pig	Day of Experiment 0 mg/ml	7 mg/ml	14 mg/ml	19 mg/ml
11.7	1	0.0011	*	*	*
	2	0.0003	*	*	*
	3	0.0006	*	*	*
	4	0.0003	0.0020	*	*
3.6	5	0.0006	0.0017	0.0037	*
	6	0.0011	0.0051	0.0023	0.0017
	7	0.0003	0.0049	0.0020	0.0011
	8	0.0003	0.0029	*	*
0.02	9	0.0006	0.0023	0.0003	0.0006
	10	0.0009	0.0006	0.0000	0.0000
	11	0.0006	0.0000	0.0009	0.0006
	12	0.0003	0.0009	0.0009	0.0003

*No results due to death of animal.

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| | | |

TABLE [illegible] -- page 2

4.5 mg/ Pic	1	0	–	–	–	–	–	–
	1	0	–	–	–	–	–	–
7.0 mg	9	0	–	–	–	–	50	314
[illegible]	12	0	–	–	–	–	50	314
[illegible]	24	0	–	–	–	–	113	314
	48	0	–	–	–	–	113	153

TABLE 4E. Skin test reactions in guinea pigs, which received intraperitoneally 5×10^8 spleen cells from Pd-egg albumin sensitive pigs, in mm^2 (erythema and induration)

			Skin tested with 0.1 ml (conc = mg/ml) intradermal injection of					
			4.5 $PdCl_4$	4.5 $PdCl_4$	3.8 $PtCl_4$	3.8 $PtCl_4$		
	Hour	Buffer	7.8 mg egg albumin	7.8 mg G-PPG albumin	7.8 mg egg albumin	7.8 mg G-PPG albumin	7.8 mg egg albumin	7.8 mg G-PPG albumin
G. pig received 5×10^8 spleen cells sensitized to Pd-egg alb.	½	0	–	–	–	–	–	–
	1	0	–	–	–	–	–	–
	4	0	314	283	–	–	–	–
	12	0	314	314	–	–	–	–
	24	0	283	314	–	–	–	–
	48	0	283	226	–	–	–	–
G. pig received 5×10^8 spleen cells sensitized to Pd-egg alb.	½	0	–	–	–	–	–	–
	1	0	–	–	–	–	–	–
	4	0	314	346	–	–	–	–
	12	0	346	380	–	–	–	–
	24	0	346	380	–	–	–	–
	48	0	283	314	–	–	–	–

TABLE 41. Skin test reactions in guinea pigs, which received intraperitoneally 5×10^8 spleen cells from Pd-g.-pig albumin sensitive pigs, in mm^2 (erythema and induration)

			Skin tested with 2.1 ml (conc = mg/ml) intradermal injection of					
			4.5 $PdCl_4$	4.5 $PdCl_4$	3.8 $PdCl_4$	3.8 $PdCl_4$		
Donor	Hours	Buffer	7.8 mg egg albumin	7.8 mg g.-pig albumin	7.8 mg egg albumin	7.8 mg g.-pig albumin	7.8 mg egg albumin	7.8 mg g.-pig albumin
C.-pig received 5×10^8 spleen cells sensitized to Pd-g.-pig albumin	½	0	–	–	–	–	–	–
	1	0	–	–	–	–	–	–
	6	0	314	314	–	–	–	–
	12	0	380	346	–	–	–	–
	24	0	380	314	–	–	–	–
	48	0	314	283	–	–	–	–
C.-pig received 5×10^8 spleen cells sensitized to Pd-g.-pig albumin	½	0	–	–	–	–	–	–
	1	0	–	–	–	–	–	–
	6	0	314	284	–	–	–	–
	12	0	314	314	–	–	–	–
	24	0	314	346	–	–	–	–
	48	0	314	285	–	–	–	–

TABLE 42. Skin test reactions (mm^2 erythema and induration) in guinea pigs sensitised with 3.7 mg/ml palladium complexed to 5.7 mg/ml egg albumin.

Guinea pigs sensitised 3.7 mg/ml Pd-5.7 mg/ml egg albumin	Hour	Skin tested with 0.1 ml (conc = mg/ml) intradermal injection of 3.7 Pd and 5.7 egg albumin
#1	½	0
	1	0
	9	176
	12	143
	24	132
#2	½	0
	1	0
	9	264
	12	176
	24	132
#3	½	0
	1	0
	9	113
	12	177
	24	132
#4	½	0
	1	0
	9	214
	12	283
	24	236
#5	½	0
	1	0
	9	113
	12	132
	24	132

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96	6	0
	9	71
	12	113
	24	104
97	6	0
	9	6
	12	0
	24	0

TABLE 41 Skin test reactions (mm^2 erythema and induration) in normal guinea pigs receiving 5×10^8 spleen cells intraperitoneally from sensitive donors.

Recipient guinea pig sensitised with cells from	Hour	Skin tested with 0.1 ml (conc = mg/ml) intradermal injection of 3.7 mg Pd and 5.7 egg albumin
G. pig #1	4	0
	8	0
Table 41	9	240
	12	226
	24	101
G. pig #2	4	0
	8	0
Table 41	9	240
	12	214
	24	200
G. pig #3	4	0
	8	0
Table 41	9	226
	12	200
	24	113
G. pig #4	4	0
	8	0
Table 41	9	314
	12	268
	24	113

All other sites and times - readings were zero.

TABLE 44. Skin test reaction (mm^2 erythema and induration) in guinea pigs sensitized with 5.0 mg/ml Pd complexed to 7.0 mg/ml egg albumin. Skin tested with 0.1 ml (conc = mg/ml)

Guinea Pig	Hour	Sensitized to 3.7 Pd		Sensitized to 5.0 Pd	
		3.7 Pd 7.0 egg alb	5.0 Pd 7.0 egg alb	3.7 Pd 7.0 egg alb	5.0 Pd 7.0 egg alb
61	½	0	0	0	0
	1	0	0	0	0
	9	226	346	268	490
	12	240	416	283	490
	24	240	379	246	397
62	½	0	0	0	0
	1	0	0	0	0
	9	254	362	268	314
	12	254	362	283	314
	24	254	362	254	314
63	½	0	0	0	0
	1	0	0	0	0
	9	254	200	240	490
	12	200	226	240	314
	24	200	200	226	314
64	½	0	0	0	0
	1	0	0	0	0
	9	70	314	177	188
	12	66	314	177	188
	24	66	314	177	188
65	½	0	0	0	0
	1	0	0	0	0
	9	166	268	213	146
	12	188	329	226	180
	24	176	329	200	314

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06	6	0	0	0	0
	1	0	0	0	0
	9	346	213	213	124
	18	346	213	213	314
	24	240	213	176	314
07	6	0	0	0	0
	1	0	0	0	0
	9	176	433	200	0
	18	153	530	200	0
	24	153	490	176	0
08	6			0	0
	1			0	0
	9			0	240
	18			0	254
	24			0	240

TABLE 45. Skin test reaction (mm^2 erythema and induration) in normal guinea pigs receiving 5×10^8 spleen cells intraperitoneally from sensitised donors.

Recipient guinea pig sensitised with cells from	Hour	Skin tested with 0.1 ml (conc = mg/ml) intradermal injection of	
		3.7 Pd-7.0 egg alb	5.0 Pd-7.0 egg alb
5.0 Pd-7.0 egg alb sensitised pig	½	0	0
	1	0	0
	9	200	254
	12	213	254
	24	165	254
5.0 Pd-7.0 egg alb sensitised pig	½	0	0
	1	0	0
	9	0	0
	12	0	0
	24	0	0
3.7 Pd-7.0 egg alb sensitised pig	½	0	0
	1	0	0
	9	94	200
	12	94	200
	24	94	188
3.7 Pd-7.0 egg alb sensitised pig	½	0	0
	1	0	0
	9	165	213
	12	165	254
	24	165	254